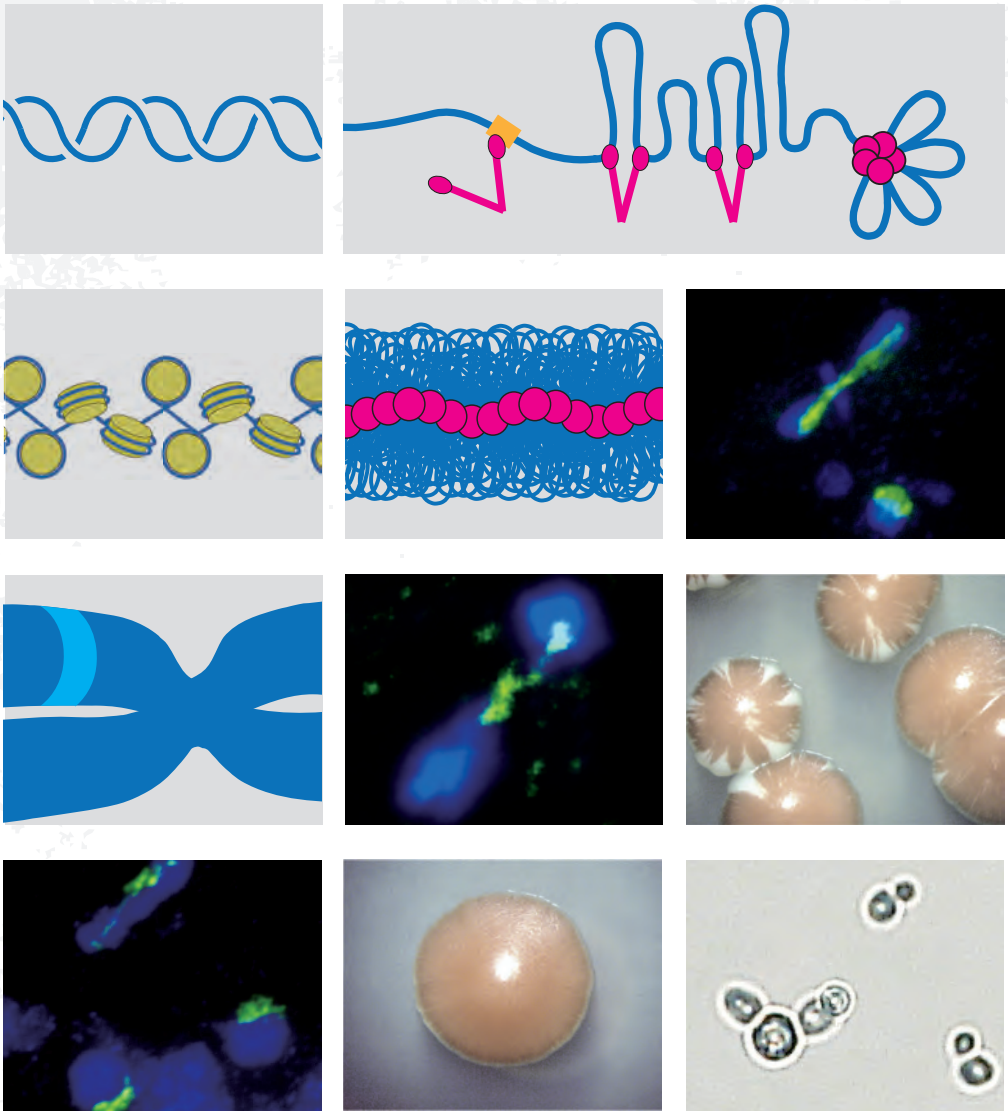


National Institute for Basic Biology

2009 ANNUAL REPORT

CONTENTS

The cover photographs and illustrations are related to the study of an initial step of chromosome condensation, namely condensin – chromatin association. The cis element and protein factors required for condensin recruitment onto chromatin have been identified from the ribosomal RNA gene (rDNA) cluster in budding yeast, *Saccharomyces cerevisiae*. Completion of chromosome segregation during mitosis requires the hypercondensation of the rDNA region during anaphase. Any defect in condensin recruitment to the cis element will result in partial condensation and non-disjunction of the chromosomal region containing an rDNA cluster. See Johzuka and Horiuchi, Mol. Cell *34*, 26-35 (2009) and page 45 of this report for detail.

Kiyotaka Okada

Our institute, the National Institute for Basic Biology (NIBB), has developed as a center of excellence in research, education, and inter-university collaboration in the various fields of basic biology since its foundation in 1977. In 2004, NIBB, together with four other national research institutes, established a new organization, the National Institutes of Natural Sciences (NINS), one of four Inter-University Research Institute Corporations. In order to carry out our mission, we have focused our efforts on five major activities, namely Promotion of Collaborative Research Projects, Promotion of Academic Research, Development of New Academic Fields, Cultivation of Future Researchers, and International Cooperation. Our works and results for 2009 are shown in this report.

In 2009, we welcomed six new colleagues, while six colleagues left the institute as shown on page 7. The results of our research groups are reported from page 8 to 74. The activities of our supporting divisions are shown on the following pages 75–84.

As international collaborative activities, we hosted the 9th NIBB-EMBL (European Molecular Biology Institute) joint meeting in Okazaki. We also participated in the 1st NIBB-EMBL student symposium in Heidelberg, and the 1st NIBB-MPIZ (Max Planck Institute for Plant Breeding Research) joint symposium in Cologne as shown on pages 85-87. In addition, we held two practical training courses with lectures and technical training. One of the courses was held for students and young researchers interested in learning about a new model plant, *Physcomitrella patens*. The other newly instituted course was held for those desiring a fundamental knowledge of microarray analysis, as reported on page 88.

In addition to research, the education of the next generation of researchers is another important aim of NIBB. As a department of the Graduate University for Advanced Studies, we are happy we have the opportunity to educate graduate students. At present, we have 39 students, as reported on page 6.

Kiyotaka OKADA, D. Sci.
Director-general, NIBB
March 24, 2010

ORGANIZATION OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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

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

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

Policy, Decision Making, and Administration

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

The Strategic Planning Department assists the director-general with NIBB's evaluation procedures and in planning a long-range strategy for the institute. The Office of Public Relations and International Cooperation is a central office for public relations and the management of conferences and other extramural activities.

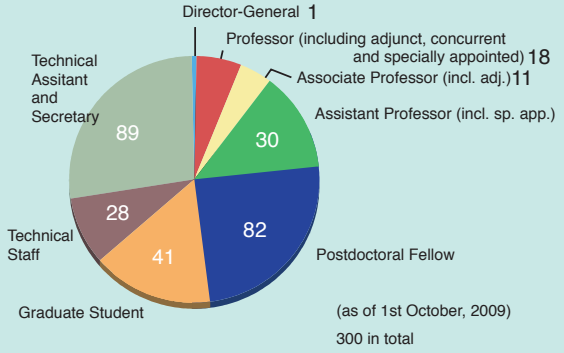
Administration of NIBB is undertaken by the Okazaki Administration Office under the direct auspices of the Administration Office of NINS.

Research and Research Support

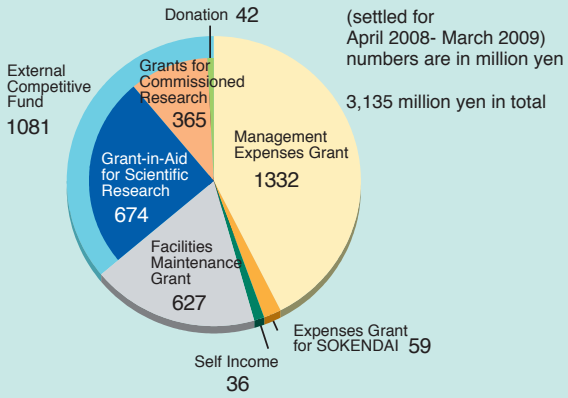
NIBB conducts its research programs through research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. Some of the research units are adjunct and led by professors who hold joint appointments with other universities. Adjunct divisions have a resident assistant professor. This arrangement facilitates exchange in research activities in Japan. The Technical Division manages the activities of the technical staff and helps to promote the activities of each research unit and to maintain the common resources of both the research support units of the NIBB and the research facilities of the Okazaki campus.

The 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 the NIBB

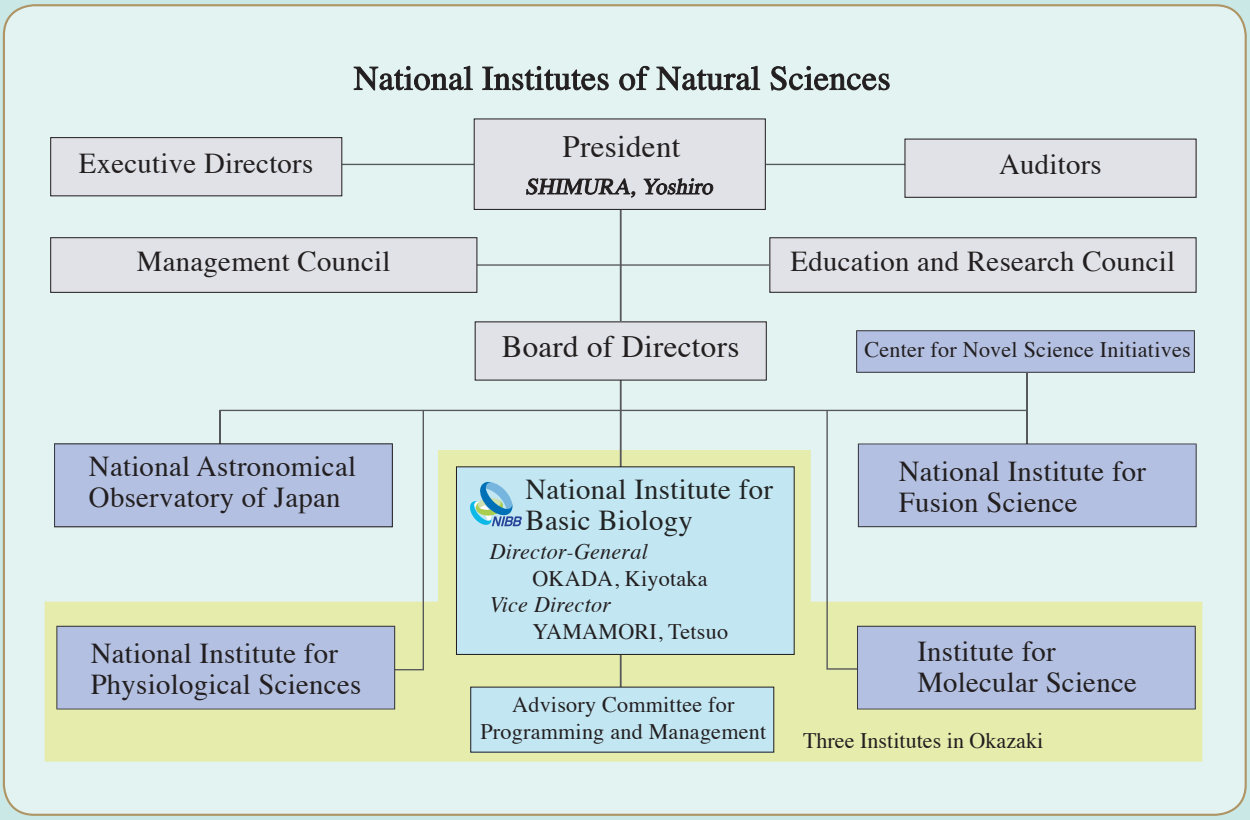


Financial Configuration of the NIBB



Organization

As of April 1, 2009



Members of the Advisory Committee for Programming and Management (as of April, 2009)

Chairperson	NODA, Masaharu	Professor, National Institute for Basic Biology
Vice-Chair	MATSUOKA, Makoto	Professor, Nagoya University
Non-NIBB members	FUKUDA, Hiroo	Professor, The University of Tokyo
	ISHINO, Fumitoshi	Professor, Tokyo Medical and Dental University
	KATO, Kazuto	Associate Professor, Kyoto University
	OSUMI, Noriko	Professor, Tohoku University
	SAKANO, Hitoshi	Professor, The University of Tokyo
	SATOH, Noriyuki	Faculty, Okinawa Institute of Science and Technology
	SIOMI, Haruhiko	Professor, Keio University
	TABATA, Satoshi	Deputy Director, Kazusa DNA Research Institute
	YANAGIDA, Toshio	Professor, Osaka University
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	HORIUCHI, Takashi	Professor, National Institute for Basic Biology
	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
	KOBAYASHI, Satoru	Professor, Okazaki Institute for Integrative Bioscience
	NISHIMURA, Mikio	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, National Institute for Basic Biology
	UENO, Naoto	Professor, Okazaki Institute for Integrative Bioscience
	YAMAMORI, Tetsuo	Professor, National Institute for Basic Biology
	YOSHIDA, Shosei	Professor, National Institute for Basic Biology

National Institute for Basic Biology

As of April 1, 2009

Research Units

Cell Biology
- Division of Cell Mechanisms
- Laboratory of Neuronal Cell Biology††††
- Laboratory of Cell Structure
- Laboratory of Cell Sociology

Developmental Biology
- Division for Morphogenesis
- Division of Developmental Genetics †
- Division of Molecular and Developmental Biology ††
- Division of Embryology
- Division of Germ Cell Biology
- Division of Reproductive Biology
- Laboratory of Molecular Genetics for Reproduction
- Laboratory of Plant Organ Development

Neurobiology
- Division of Molecular Neurobiology
- Division of Brain Biology
- Laboratory of Neurophysiology
- Laboratory of Neurochemistry

Evolutionary Biology and Biodiversity
- Division of Genome Dynamics
- Division of Evolutionary Biology
- Division of Symbiotic Systems
- Laboratory of Morphodiversity
- Laboratory of Bioresource

Environmental Biology
- Division of Molecular Environmental Endocrinology †††
- Division of Plant Developmental Genetics (adjunct)
- Laboratory of Photoenvironmental Biology

Theoretical Biology
- Division of Theoretical Biology
- Laboratory of Genome Informatics

Imaging Science
- Division of Developmental Dynamics (adjunct)
- Laboratory for Spatiotemporal Regulations

Research Support Units

Research Support Facilities
- The Large Spectrograph Laboratory
- Tissue and Cell Culture Laboratory
- Computer Laboratory
- Plant Culture Laboratory

Center for Transgenic Animals and Plants

Research Center for Integrative and Computational Biology

Other Research Support Sections

Technical Division

Strategic Planning Department

Office of Public Relations and International Cooperation

Okazaki Research Facilities

Okazaki Institute for Integrative Bioscience
- Department of Development, Differentiation and Regeneration
  - Division of Developmental Genetics *
  - Division of Molecular and Developmental Biology **
- Department of Bio-Environmental Science
  - Division of Bio-Environmental Science ***
  - Division of Neuronal Cell Biology ****
- Department of Strategic Methodology

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

Center for Radioisotope Facilities

Center for Experimental Animal

Research Center for Computational Science

Research Facilities run jointly with National Institute for Physiological Sciences

Center for Analytical Instruments
- Laboratory Glassware Facilities
- Electron Microscope Center
- Machine Shop
- Low-Temperature Facilities

GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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

Promotion of Collaborative Research

Collaborative Research Projects

Research projects in collaboration with NIBB's divisions/ laboratories and research activities conducted using NIBB's facilities are solicited from external researchers at other universities and institutes. In addition to conventional "individual collaborative research projects," "collaborative experiments using the Large Spectrograph," and "NIBB workshops," new types of research projects are solicited that facilitate the strategic organization of collaborative research projects. "Priority collaborative research projects" are carried out as group research by internal and external researchers with the purpose of developing pioneering research fields in biology. Five projects have already been carried out and as an outcome of the project "Molecular mechanisms for controlling the individuals of higher plants," Prof. I. Terashima (Univ. Tokyo) started a project of Grant-in-Aid for Scientific Research on Innovative Areas in 2009. The category "Collaborative research projects for model organism/technology development" was started in 2007. Six projects have already been carried out, including those using *Cabombaceae* or *Pophyra* as model organisms and those related to methdologies in medaka genome analyses. NIBB always encourages discussion on such projects in the belief that our methods of conducting collaborative research projects must be constantly modified, according to the demands of the age and the biology community.

Collaborative research projects by year

year	2006	2007	2008	2009
Priority collaborative research projects	3	1	0	1
Collaborative research projects for model organisms/ technology development	–	2	3	2
Individual collaborative research projects	37	43	49	54
NIBB workshops	1	5	5	3
Collaborative experiments using the Large Spectrograph	18	14	11	10
Facility Use (Center for Analitical Instruments)	0	1	0	0
total	59	66	68	71

Enhancement of the Large Spectrograph

The Large Spectrograph Laboratory, a world-leading research facility in photobiology, has been instrumental in a large number of collaborative research projects since its establishment in 1980. To ensure further high-level achievements, the Laboratory has undertaken an enhancement of its laboratory equipment, including the addition of advanced control systems, and the use of laser sources and sophisticated analysis equipment. To supplement the currently operating fixed (partly tunable) wavelength laser sources, we are preparing to introduce tunable laser sources covering a wide range of wavelengths from UV to IR.

International Cooperation

Collaborative Programs with EMBL

The European Molecular Biology Laboratory (EMBL), established in 1974, is a research institute funded by 18 European nations. It conducts comprehensive, high-level basic research programs and leads the world in the field of molecular biology. NIBB takes a leading role in the collaborative research programs between EMBL and the National Institutes of Natural Sciences (NINS), which were launched in 2005, and promotes personal and technological exchange through symposia, exchange between researchers and graduate students, and the introduction of experimental equipment (see page 85 for details of the EMBL meetings held in 2009).



Meeting (left) and discussions (right) in EMBL, Heidelberg

Collaborative Programs with MPIZ

NIBB formed an agreement with the Max Planck Institute for Plant Breeding Research (MPIZ) in April 2009 to start a new initiative aimed at stimulating academic and scholarly exchange in the field of plant sciences. NIBB and MPIZ work together to plan and promote joint research projects, collaborative symposia, training courses and student exchange programs. NIBB acts as a bridge between Japanese and German researchers in the field of plant sciences. (see page 87 for details of the joint meeting held in 2009).

NIBB Conference

The NIBB Conference is an international conference organized by NIBB's professors once or twice a year with the participation of guest lecturers from abroad. Since the first conference in 1977 (the year of NIBB's foundation), the NIBB Conference has provided researchers in basic biology with valuable opportunities for international exchange.

International Practical Course

With the cooperation of researchers from Japan and abroad the NIBB international practical course, a practical training program, is given at a laboratory specifically prepared for its use at NIBB. The fourth course was held in 2009 (see page 88). Graduate students and young researchers from various areas including EU countries, Israel, India, and Singapore, were provided with training in state-of-the-art research techniques.

Bio-Resources

The National BioResource Project (NBRP) is a national project for the systematic accumulation, storage, and supply of nationally recognized bio-resources (experimental animals and plants, cells, DNA, and other genetic resources), which are widely used as materials in life science research. To promote this national project, NIBB has been appointed as a center for research on "Medaka (*Oryzia latipes*)" whose usefulness as a vertebrate model first developed in Japan. 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 and Zebrafish. In addition, NIBB provides databases containing research data on the moss *Physcomitrella patens*, *Daphnia*, *Xenopus laevis*, plant cell organelles, and bacterial genomes.

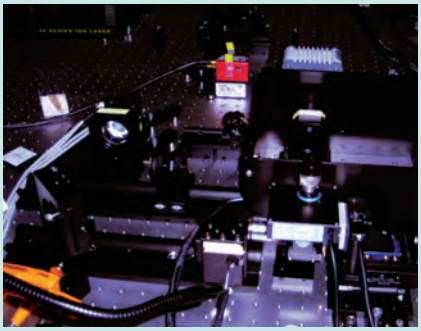
Top: Medaka strain for which the full genome sequence was determined,
Middle: Transgenic Medaka with red fluorescence,
Bottom: A strain with transparent body.

Development of New Fields of Biology

Bioimaging

Recently, the capability of optical microscopes has greatly improved and biophotonic probes have been developed. The combination of these technologies allows us to use living samples and observe biological phenomena in real time, which, in the past, could only be estimated based on fragmentary information from fixed samples. NIBB aims to maximize the application of these techniques for visualizing biological phenomena (bioimaging) and to develop new imaging techniques.

1) *Imaging Science Laboratories,* NIBB aims to be a center for the development of new microscopes and biophotonic probes.
2) *Advisory Committee on Bioimaging,* Regular meetings are held with several leading researchers in the bioimaging field in Japan to formulate advice on imaging research.
3) *Bioimaging Forum,* This provides an opportunity for researchers at NIBB, members of the Advisory Committee, and company engineers to frankly discuss practical difficulties and needs regarding imaging.
4) *Introduction of DSLMs (Digital Scanned laser Light sheet Microscope),* As part of our collaborative work with EMBL, NIBB has introduced a DSLM, which is effective for the three-dimensional observation of living samples, and is the first of its kind in Japan.
5) *Bioimaging Symposium,* This provides an opportunity for academic exchange with cutting-edge overseas researchers in the imaging field, mainly from EMBL.



Optical path of the DSLM

Okazaki Biology Conferences

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

Cultivation of Future Researchers

NIBB sponsors two education programs for graduate students.

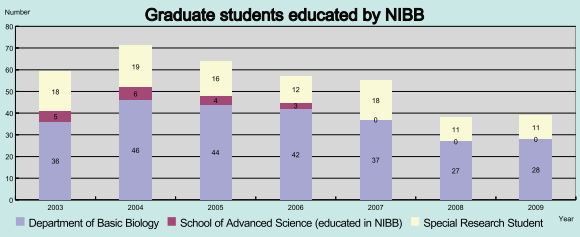
1. Graduate University for Advanced Studies

   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.

2. Graduate Student Training Program

   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.



Personnel changes in 2009*

Newly assigned in NIBB

Name	Position	Research Unit	Date
SHIINA, Nobuyuki	Associate Professor	Laboratory of Neuronal Cell Biology	March 1
KAWAGUCHI, Masayoshi	Professor	Division of Symbiotic Systems	April 1
KITADATE, Yu	Assistant Professor	Division of Germ Cell Biology	April 1
MIYAGAWA, Shin-ichi	Assistant Professor	Division of Molecular Environmental Endocrinology	June 1
SATO, Masanao	Assistant Professor	Division of Developmental Genetics	August 1
TAKEDA, Naoya	Assistant Professor	Division of Symbiotic Systems	October 1

Newly affiliated in other universitites and institutes

Name	New Affiliation	Position	Date
IIDA, Shigeru	Tokyo University of Agriculture and Technology/ University of Shizuoka	Guest Professor	April 1
OHSUMI, Yoshinori	Tokyo Institute of Technology	Professor	April 1
KATSU, Yoshinao	Hokkaido University	Associate Professor	April 1
SUZUKI, Kuninori	Tokyo Institute of Technology	Assistant Professor	April 1
NAKATOGAWA, Hitoshi	Tokyo Institute of Technology	Assistant Professor	April 1
WATANABE, Hajime	Osaka University	Professor	June 1

* Changes in professors, ascociate and assistant professors are shown.

Awardees in 2009

Name	Position	Award
OHSUMI, Yoshinori	Professor	The Asahi Prize
OKADA, Kiyotaka	Director-General	The Research Award of the Botanical Society of Japan
KUROKAWA, Hiromi	Graduate Student	DGD (Development, Growth & Differentiation) Award
TANAKA, Minoru et al.	Associate Professor	Awarded Publication: Kurokawa et al. DGD *48*, 209-, 2006
SHINTANI, Takafumi	Assistant Professor	The JB (Journal of Biochemistry) Prize
NODA, Masaharu	Professor	Awarded Publication: Shintani and Noda JB *144*, 259-, 2008

Note: On the unit member lists from P.8 to P.74 : all members who belonged to the unit during 2009 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 2009. The former title is indicated by an asterisk (*).

DIVISION OF CELL MECHANISMS

Professor
NISHIMURA, Mikio

Associate Professor
HAYASHI, Makoto

Assistant Professors:	*MANO, Shoji*
	YAMADA, Kenji
Technical Staff:	*KONDO, Maki*
NIBB Research Fellow:	*OIKAWA, Kazusato*
Postdoctoral Fellows:	*ARAI, Yuko*
	KAMIGAKI, Akane
	SHIRAYA, Takeshi
	*OIKAWA, Kazusato**
Graduate Students:	*OGASAWARA, Kimi*
	KANAI, Masatake
	GOTO, Shino
	NAKAI, Atsushi
	CUI, Songkui
	SHIBATA, Michitaro
Technical Assistants:	*NAKAMORI, Chihiro*
	YOSHINORI, Yumi
	SUZUKI, Iku
	FUKAZAWA, Mitsue
	KATO, Kyoko
	NISHINA, Momoko
	SATO, Yori
	ARAKI, Masami
	TSUCHIYA, Chiaki
	SAITO, Miyuki
Secretaries:	*UEDA, Chizuru*
	KUBOKI, Yuko

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 of Priority Areas on "Organelle Differentiation as the Strategy for Environmental Adaptation in Plants" has just finished. The project has been accumulating evidence of the mechanism of organelle differentiation and has revealed the relationship between organelle plasticities and integrated functions in plants.

I. Reversible transformation of plant peroxisomes

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

II. Transcriptomics, proteomics and phenomics of plant peroxisomes

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

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

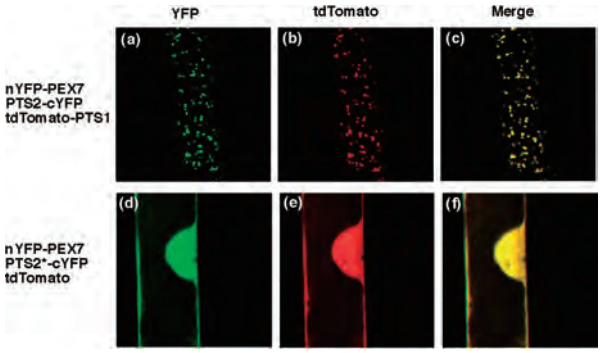


Figure 1 Detection of interaction between PEX7 and PTS2-containing proteins using BiFC. (a) Epidermal cells expressing nYFP-PEX7 and PTS2-cYFP. YFP fluorescent signal was colocalized (c) with peroxisome marker tdTomato-PTS1 (b). (d) Epidermal cells expressing nYFP-PEX7 and PTS2($R^{16}G$)-cYFP, which has inactive PTS2 by amino acid substitution from the 16th Arg to Gly. YFP signal was merged (f) with cytosolic marker, tdTomato (e).

Bioinfomatic analysis of the *Arabidopsis* genome predicted the presence of 15 kinds of genes, called *PEX* genes, for peroxisomal biogenesis factors. We demonstrated that *PEX5* and *PEX7* form a cytosolic receptor complex and recognize

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

PTS1- and PTS2-containing proteins, respectively (Figure 1). *PEX14* is a peroxisomal membrane docking protein that captures the receptor-cargo complex. We also comprehensively investigated whether or not these predicted *PEX* genes function in peroxisome biogenesis by generating knock-down mutants that suppress *PEX* gene expression by RNA-interference. Phenotypes of these mutants allowed us to identify the functional *PEX* genes, which can be classified into two groups: *PEX* genes regulating for peroxisomal protein import and *PEX* genes regulating for peroxisomal morphology. We continue to investigate the detailed molecular functions of other *PEX* genes. Of these, we proposed that *PEX10* is essential for the maintenance of ER morphology and for biosynthesis of cuticular wax.

III. Identification of novel components essential for peroxisome biogenesis

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

Of these *apm* mutants, *APM1* gene (whose defect causes the elongation of peroxisomes and mitochondria) encodes dynamin-related protein 3A (DRP3A), one member of the dynamin family. In *apm2* and *apm4*, the GFP fluorescence is observed in the cytosol as well as in peroxisomes, showing the defect of protein transport to peroxisomes. We demonstrated that *APM2* and *APM4* encode proteins homologous to PEX13 and PEX12, respectively, and that APM2/PEX13 and APM4/PEX12 are components of the protein-translocation machinery on peroxisomal membranes. We are currently analyzing the functions of other APM proteins. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, and to address the mechanism at the molecular level.

IV. ER derived organelles for protein storing and defense strategy

Plant cells develop various types of endoplasmic reticulum (ER)-derived structures with specific functions. ER bodies are ER-derived compartments observed in *Arabidopsis*. They are rod-shaped structures surrounded by ribosomes. ER bodies are widely distributed in the epidermal cells of whole seedlings. Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid treatment. This suggests that ER bodies function in the defense against herbivores. ER bodies in seedlings include PYK10, a β-glucosidase with an ER retention signal. *Arabidopsis nai1* mutants have no ER bodies in the entire plant and do not accumulate PYK10. *NAI1* encodes a transcription factor that has a basic-helix-loop-helix (bHLH) domain and regulates the expression of *PYK10*. *Arabidopsis nai2* mutant has no ER bodies and reduces the accumulation of PYK10. *NAI2* encodes a unique protein that localizes to the ER body. We found that the membrane protein of ER body 1 (MEB1) and MEB2 are integral membrane proteins of the ER body (Figure 2). NAI2 deficiency relocates MEB1 and MEB2 to the ER network. These findings indicate that NAI2 is a key factor that enables ER body formation.

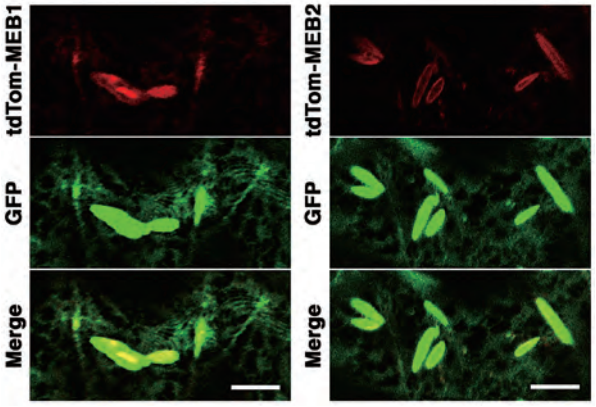


Figure 2. MEB1 and MEB2 localize to the ER body membrane. Fluorescence images of cotyledon epidermal cells transiently expressing tdTom-MEB1 fusion protein (left) or tdTom-MEB2 (right) in a 7-day-old *Arabidopsis* transgenic plant that expresses ER-targeted GFP.

V. Vacuoles responsible for programmed cell death in plants

The vacuolar processing enzyme (VPE) belongs to the cysteine protease family found in higher plants and animals. VPE is responsible for the maturation of various types of vacuolar proteins. We revealed a novel function of VPE in various instances of programmed cell death (PCD) in plants. VPE is identified as the proteinase that exhibits caspase-1 activity in plants. The plant hypersensitive response (HR), a type of defense strategy, constitutes well-organized PCD. No HR occurs on the tobacco mosaic virus-infected leaves of VPE-deficient tobacco plants. These results suggest that VPE is involved in vacuolar collapse, which triggers PCD.

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

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

VI. Roles of molecular chaperones on cell differentiation

Molecular chaperones are cellular proteins that function in the folding and assembly of certain other polypeptides into oligomeric structures. To clarify the roles of molecular chaperones on cell differentiation, we have purified and characterized chaperonin and Hsp70s and analyzed their roles in the translocation of proteins into chloroplasts.

Recently, we found that HSP90 inhibitor induced genes with heat shock response element (HSE) motifs in their

promoters, suggesting that heat shock transcription factor (HSF) is involved in the response. *Arabidopsis* HSFs interacted with HSP90.2. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. During heat shock, HSP90 is transiently inactivated, which leads to HSF activation. This data indicates that HSP90 regulates correct gene expression for heat acclimatization in plants. We also observed that HSP90 is involved in hormone responses in *Arabidopsis*. The evolutional and functional characterizations are now being investigated.

VII. The Plant Organelles Database 2 (PODB2) – Release of version 2 for plant organelles dynamics and methods for functional analysis

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 4 individual parts: the organelles movie database, the organellome database, the functional analysis database, and external links. The organelles movie database contains videos of organelle movements and 3D structures (Figure 3). The organellome database provides images of various plant organelles that were visualized with fluorescent and nonfluorescent probes in various tissues of several plant species at different developmental stages. The functional analysis database is a collection of protocols for plant organelle research. The amount of included data is increasing day by day. It is expected that PODB2 will contribute to systems biology through the combination of the included data with other 'omics' data and computational analyses. In addition, we will release an updated version for educational use soon. We expect that PODB2 will be a useful tool to help researchers gain greater knowledge of plant organelles, as well as the general public who want to explore plant cell biology.

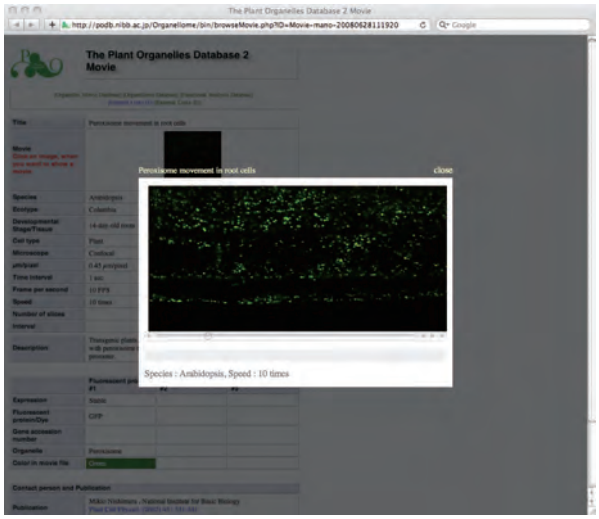


Figure 3. The graphical user interfaces of 'Organelles Movie Database' in PODB2 (http://podb.nibb.ac.jp/Orgenellome).

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DIVISION OF MOLECULAR CELL BIOLOGY †

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Most cellular activity is maintained by the balance between the synthesis and degradation of related proteins. Degradation processes, therefore, play important roles in many physiological aspects as well as in the regulation of gene expression. Autophagy is a bulk degradation system for cytosolic proteins and organelles in lysosomes/vacuoles that is highly conserved in eukaryotic cells. Our division aims to understand the physiological roles and molecular mechanisms of autophagy in yeast and higher eukaryotes.

I. Background

In response to nutrient starvation, the autophagic process starts as a building up of membrane structures called autophagosomes in the cytoplasm. The autophagosome sequesters a part of the cytosol and organelles non-selectively. It is then delivered to the vacuole/lysosome, and the cytoplasmic materials inside are degraded by vacuolar/lysosomal hydrolases. We discovered autophagy in a simple model organism, the budding yeast *Saccharomyces cerevisiae,* and have morphologically and genetically defined the whole process.

II. Mitochondria-specific autophagy requires the transmembrane receptor Atg32

Autophagy-dependent degradation of mitochondria is a fundamental process conserved from yeast to humans. In contrast to starvation-induced, nonselective autophagy responsible for nutrient recycling, selective autophagy, which involves particular cues and receptors required for induction and cargo recognition, respectively, mediates mitochondria-specific breakdown. Although numerous studies highlight that mitochondria-specific autophagy (mitophagy) contributes to mitochondrial homeostasis, the molecular mechanisms underlying this selective clearance process are very poorly understood. We establish that a substantial fraction of mitochondria are exclusively sequestered and transported to the vacuole, a lytic compartment, in post-log phase cells under respiratory conditions (Figure 1). Using a genome-wide visual screen, we identified Atg32, a protein essential for mitophagy in budding yeast. During respiratory growth, Atg32 is highly expressed, likely in response to oxidative stress, and anchored on the surface of mitochondria. We also demonstrate that Atg32 interacts with Atg8 and Atg11, proteins critical for recognition of cargo receptors. Notably, Atg32 contains WXXI/L/V, a conserved motif that serves as a binding site for the Atg8 family members. Atg32 exposes its N- and C-terminal domains to the cytosol and mitochondrial intermembrane space, respectively. We propose that Atg32 is a transmembrane receptor that directs autophagosome formation to mitochondria.

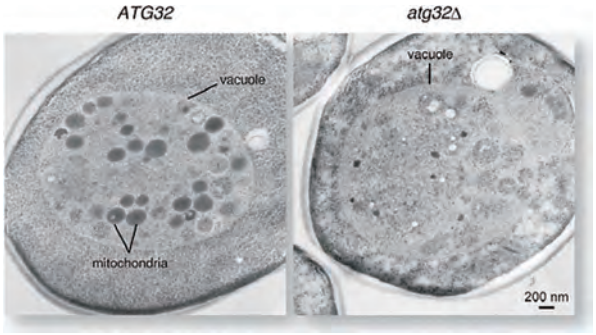

Figure 1. Electron microscopy reveals electron-dense bodies containing mitochondria accumulated in vacuoles under respiratory conditions. Vacuolar protease-deficient cells were grown in non-fermentable medium for 5 days. Notably, mitochondria are selectively incorporated into the vacuolar lumen in an Atg32-dependent manner.

III. A novel physiological role of autophagy in plants: plant autophagy puts the brakes on cell death by controlling salicylic acid signaling

It has long been thought that autophagy in plants is important for nutrient recycling and plays a critical role in the ability of plants to adapt to environmental variation such as nutrient deprivation. Recent reverse genetic studies, however, hint at other roles for autophagy, showing that autophagy defects in higher plants result in early senescence and excessive immunity-related programmed cell death (PCD), irrespective of nutrient conditions. Until now the mechanisms by which cells die in the absence of autophagy were unclear. In collaboration with RIKEN Plant Science Center research groups, using biochemical, pharmacological and genetic approaches, we revealed a conserved requirement for salicylic acid (SA) signaling for these phenomena in autophagy-defective mutants (*atg* mutants). The *atg* mutant phenotypes of accelerated PCD in senescence and immunity were suppressed by inactivation of SA signaling but not by inactivation of jasmonic acid (JA) or ethylene (ET) signalings (Figure 2). Application of an SA agonist restored the senescence/cell death phenotype in

†: This laboratory was closed on 31 March, 2009.

SA-deficient *atg* mutants but not in *atg npr1* plants, suggesting that the cell death phenotypes in the *atg* mutants are dependent on the SA signal transducer NPR1. We also showed that the SA signal can induce autophagy in plants. These findings demonstrate a novel physiological function for plant autophagy that operates a negative feedback loop to modulate SA signaling.

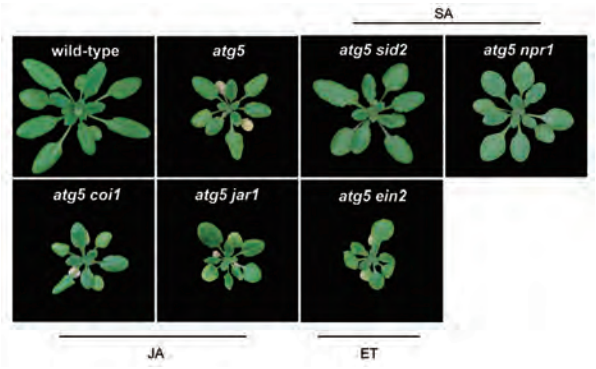

Figure 2. Accelerated PCD phenotypes in autophagy-defective plants are SA signaling dependent but do not require intact JA and ET signaling pathways. The early senescence phenotype of *atg5* mutant was suppressed by reducing SA biosynthesis in *sid2* mutant background, or by blocking SA signaling in *npr1* mutant background. Shown are photographs of five-week-old plants grown on rockwool supplied with a rich nutrient solution under long-day conditions.

IV. Characterization of the Atg17-Atg29-Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*

In the yeast, *Saccharomyces cerevisiae*, Atg17, Atg29, and Atg31/Cis1 are specifically required for autophagosome formation by acting as a scaffold complex essential for pre-autophagosomal structure (PAS) organization. We show that these proteins constitutively form an Atg17-Atg29-Atg31 ternary complex, in which phosphorylated Atg31 is included (Figure 3). Reconstitution analysis of the ternary complex in *E. coli* indicates that the three proteins are included in equimolar amounts in the complex. The molecular mass of a monomeric Atg17-Atg29-Atg31 complex is calculated at 97 kDa; however, analytical ultracentrifugation shows that the molecular mass of the ternary complex is 198 kDa, suggesting a dimeric complex. We propose that this ternary complex acts as a functional unit for autophagosome formation.

V. Structural studies of Atg protein conjugation systems

Atg proteins contain two ubiquitin-like protein conjugation systems. In collaboration with Dr. Inagaki's group at Hokkaido University, understanding of these systems has proceeded from the viewpoint of structural biology. The ubiquitin-like protein Atg8 is synthesized as a precursor form with an arginine at the C terminus, which is immediately removed by the cysteine protease Atg4 to expose a glycine residue at the new C terminus. Then, this glycine of Atg8 is conjugated to the lipid PE (phosphatidylethanolamine) via reactions catalyzed by Atg7 and Atg3, E1 and E2 enzymes, respectively. Atg4 also serves as a deconjugation enzyme for Atg8; it cleaves the Atg8-PE conjugate and releases Atg8 from membranes. The crystal structure of LC3 (a mammalian Atg8 homolog) complexed with Atg4B (a mammalian Atg4 homolog) has been determined. This provided insights into

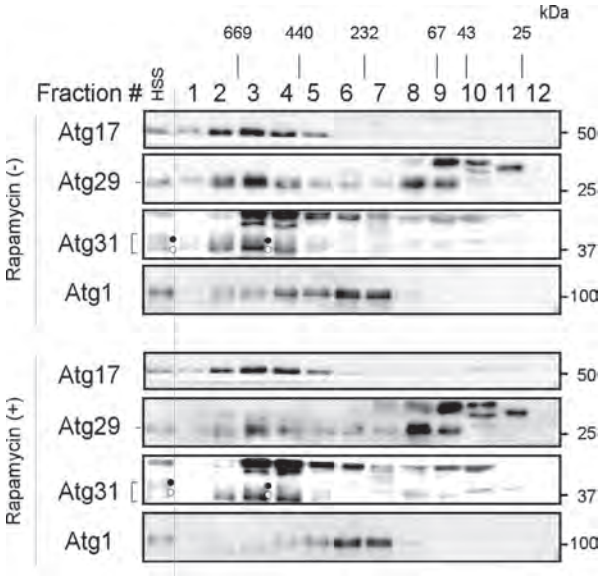

Figure 3. Atg17, Atg29, and Atg31 form a stable complex *in vivo*. Wild-type cells (BY4741) were grown and treated with rapamycin for 1 h. After cells were converted to spheroplasts, cell lysates were prepared by osmotic lysis. Cytosolic fractions (100,000 x *g* supernatant, HSS) were separated by size exclusion chromatography on a Superdex 200 column. Each fraction was analyzed by immunoblotting using anti-Atg1, anti-Atg17, anti-Atg29, and anti-Atg31antibodies. Positions of molecular mass standards (in kDa) are shown. Open and closed circles indicate dephosphorylated and phosphorylated Atg31, respectively.

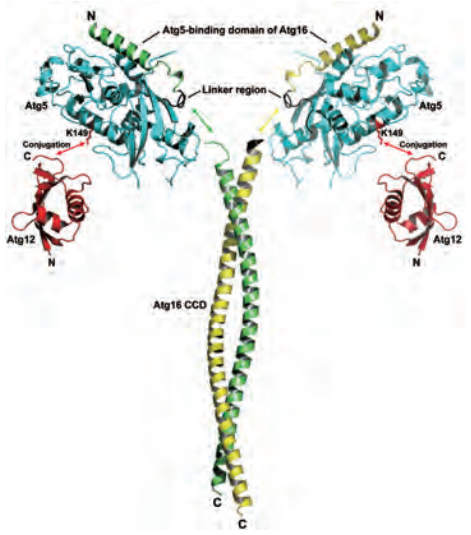

Figure 4. Model of the Atg12-Atg5-Atg16 complex.

the mechanisms of the C-terminal processing and delipidation of Atg8 by Atg4, especially for molecular recognition and regulation in these cleavage reactions.

We previously showed that the conjugation reaction of Atg8 is accelerated by a conjugate formed by the other ubiquitin-like protein Atg12 and its target protein Atg5. The Atg12-Atg5 conjugate further forms a complex with Atg16, which is required for the localization of this complex to the PAS. The crystal structure of Atg16 has been solved, which revealed that Atg16 forms a parallel coiled-coil dimer. Together with the previously-reported structures of Atg12 and Atg5, the entire architecture of the Atg12-Atg5-Atg16 complex was modeled (Figure 4). This provided an important structural basis to further elucidate the function of the complex and the mechanism of autophagosome formation.

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Laboratory of Neuronal Cell Biology

Associate Professor
SHIINA, Nobuyuki

Our laboratory was started in March 2009. Our main interest is to understand the mechanisms and roles of mRNA transport and local translation in neuronal dendrites using mice.

The transport of specific mRNAs and local control of translation 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 laboratory is researching factors regulating mRNA transport and local translation, their target mRNAs, and the mechanisms of localized protein synthesis in order to better understand its relation to the formation of synapses and neural networks, memory, learning, and behavior.

I. Identification and characterization of components of local translational machinery

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

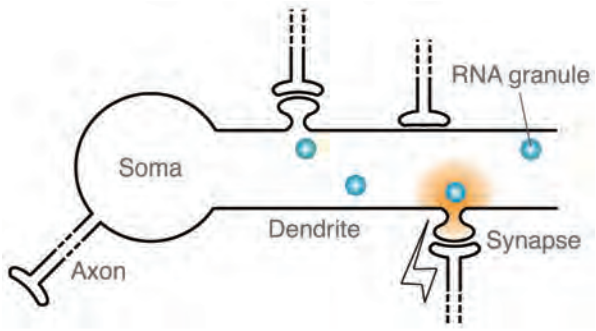


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

We have identified RNG105, an RNA-binding protein, as a component of RNA granules. RNG105 dissociates from RNA granules after synaptic stimulation, which is accompanied by the induction of mRNA translation near the granules, suggesting its involvement in local translation. Furthermore, we have generated RNG105 knockout mice and shown that RNG105 deficiency affects the formation and maintenance of synapses and neuronal networks (Figure 2). We are further studying the molecular mechanism underlying the RNG105 knockout phenotype by identifying and characterizing RNG105-associated mRNAs and proteins.

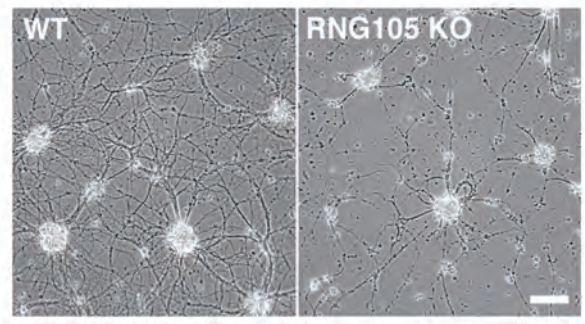


Figure 2. Phase contrast images of primary cultured neurons from wild-type (WT) and RNG105 knockout (KO) fetal brains. RNG105 knockout neurons formed poor networks compared to wild-type neurons. Scale bar, 100 μm.

We have also identified an RNG105 paralog, RNG140. RNG105 and RNG140 were localized to distinct RNA granules and showed different expression pattern and subcellular localization. Particularly, RNG105 was highly expressed in the brain of embryos, whereas RNG140 was highly expressed in the brain of adults. These results suggest that different kinds of RNA granules function in distinct neuronal cell types at distinct developmental stages.

II. Mechanisms regulating local translation in dendrites

RNA granules are densely packed structures and translationally dormant during transport, but they become less compact during conversion into translating polysomes after synaptic stimulation. We will study the mechanisms regulating the stimulation-dependent local translation by analyzing translational regulation of RNG105-associated mRNAs in neuronal dendrites.

III. Roles of local translation in higher order brain functions

RNG105 knockout neonates were born normally but died soon after birth because of respiratory failure, suggesting defects in the brainstem functions of RNG105 knockout mice. Because of the neonatal lethality, analyses of RNG105 roles have been limited to embryonic brains and neurons. In order to examine the roles of RNG105 in memory, learning, and behavior in adult mice, we are generating conditional RNG105 knockout mice. We will further generate knockout mice for RNG140 and RNG105- and RNG140-associated mRNAs and proteins to analyze their roles in higher order brain functions.

LABORATORY OF CELL STRUCTURE

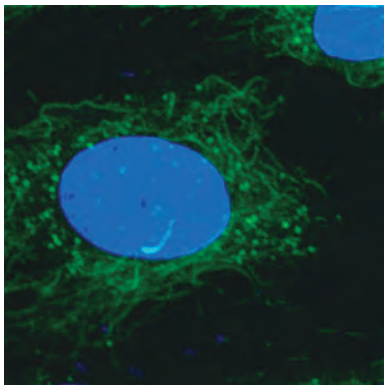
Associate Professor
OGAWA, Kazuo

Numerous proteins localized in the cilia are linked to human diseases such as PCD (primary ciliary dyskinesia) and PCKD (polycystic kidney disease). Primary cilia are defined as single cilia that grow out of one of the centrioles during interphase in otherwise unciliated animal cells. They show a 9+0 pattern, losing a central pair of microtubules, contrasting with motile cilia with a well-known 9+2 pattern.

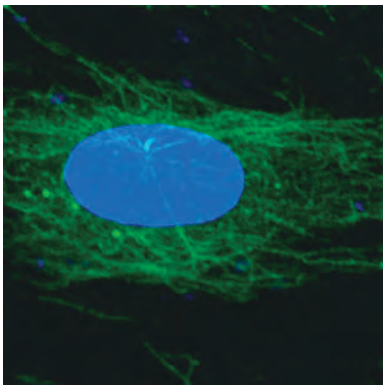
These cilia can be seen in cultured cells such as 3T3, 3T6, BHK21, NRK, and MDCK. They are quite common centriolar specializations in vivo and in vitro. The incidence of primary cilia within a cell culture is related to the degree of confluency. Examination of confluent cell monolayers showed that the primary cilia within a single preparation of a given cell line varied considerably in length. In most cases, cell lines previously used were not cloned and the results remained obscure.

Four cell lines originating from adult mouse kidneys were established in order to study the proteomics of the primary cilia. The cell lines were named nibb-K1, K4, K5, and K8. The primary cilia of the cells were observed by indirect immunofluorescence microscopy (Figure 1). In the cloned cells, each cell has a distinct length of cilium, with the K5 cell having the longest one among them (up to 10 μm).

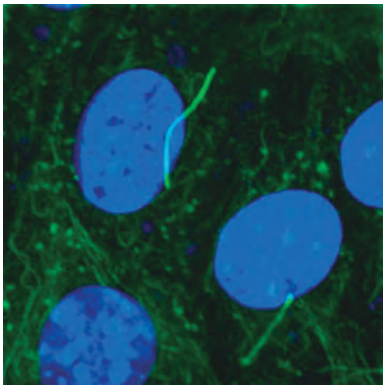
This year, we studied the relationship between primary cilia formation and one isotype of the dynein family using these cells.



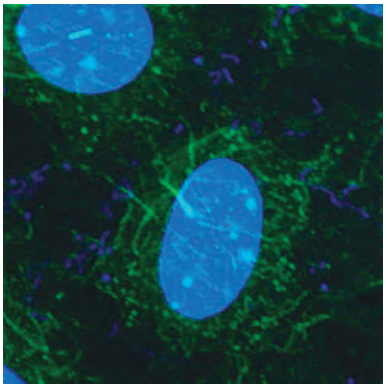
nibb-K1



nibb-K4



nibb-K5



nibb-K8

Figure 1. Typical primary cilia of four cell lines. Cells cultured on the cover slips were reacted with anti-acetylated tubulin antibody, followed by FITC-labeled secondary antibody. DNA was stained with DAPI.

LABORATORY OF CELL SOCIOLOGY

Assistant Professor
HAMADA, Yoshio

Technical Assistants: *GONDA, Naoko*
TAKAYANAGI, Noriko

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

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

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

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

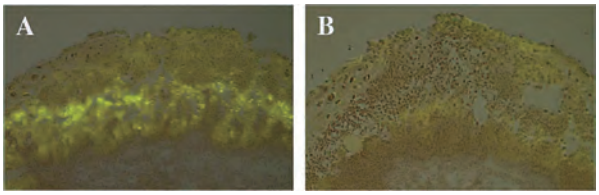


Fig.1 Programmed cell death in the developing mouse placenta. Dying trophoblast was visualized by staining with a fluorescent dye at E9.5. Wild type placenta showed extensive cell death around newly forming maternal blood beds which were surrounded by Notch2 expressing trophoblast (A), but cell death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

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The complex morphogenesis of organisms is achieved by consecutive cell-to-cell interactions during development. Recent studies suggest that growth factors play crucial roles in controlling such intercellular communications in a variety of organisms. In addition to secretory factors that trigger intracellular signaling, transcription factors that act in the nucleus to regulate gene expression are thought to be essential for the determination of cell fates. Our main interest is to know how pattern formation and morphogenesis during development is regulated by these growth and transcription factors. We address this problem using several model animals, including frogs, mice and ascidians, and by employing embryology, genetics, molecular and cellular biology, and biochemistry.

I. Establishment of cell polarity during vertebrate embryogenesis

Gastrulation is one of the most important processes during the morphogenesis of early embryos, involving dynamic cell migration and change in embryo shape. In spite of its importance, the mechanism underlying the event has just begun to be studied at the molecular level. During *Xenopus* gastrulation, mesodermal cells migrate to the inside of the embryo and move on the blastocoel roof. One of the important mechanisms for this process is the cell movement called “convergent extension (CE)”. As convergent extension begins, cells are polarized and aligned mediolaterally, followed by the mutual intercalation of the cells that acquired planar cell polarity (PCP). In the regulation of vertebrate convergent extension, the Wnt/PCP pathway is implicated.

We have recently knocked out one of two *prickle*-related genes, *mpk1*, in mice and found that the *mpk1*$^{-/-}$ mutants are early embryonic lethal and die between E5.5 and E6.5. The mutants show arrested development with failure of primitive streak and mesoderm formation and failure of distal visceral endoderm migration. At the cellular level, the *mpk1*$^{-/-}$ epiblast tissue is disorganized, with a clear defect in cell polarization.

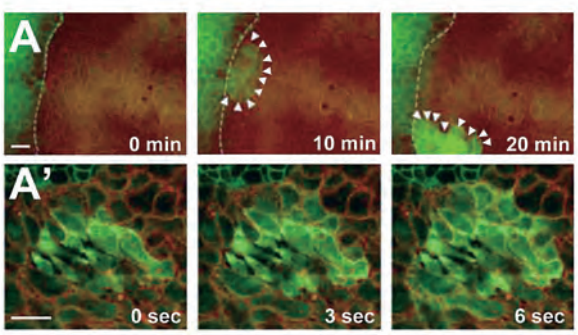

Figure 1. Intracellular Ca^{2+} increase at the border of mesoderm and ectoderm in the conjugate culture. During the culture, several islands of transiently elevated Ca^{2+} detected by a calcium indicator G-CaMP4 were observed in the single microscopic field (A). The Ca^{2+} islands appeared to be formed by a propagation of Ca^{2+} wave from fewer cells with higher Ca^{2+} levels (A’).

We also study how PCP is established within the cells using explants of *Xenopus* embryonic tissues and found that heterogenous combination culture of tissues such as mesoderm and ectoderm triggers the cell polarity, as revealed by the live-imaging analysis of microtubule growth orientation. We have been able to demonstrate that intracellular Ca^{2+} is increased prior to the oriented growth of microtubules (Figure 1), and the transient Ca^{2+} increase can be triggered not only by tissue-tissue interactions, as we reported previously, but also by physical forces experimentally applied (A. Shindo et al., PLos ONE, 5, e8897, 2010). We propose that mechanical forces generated by the tissue-tissue interactions are essential for the initiation of the cell polarity and are currently investigating to identify molecules involved in the sensing of the physical forces and transmission of the signal to the cells.

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

Although Wnt/PCP signaling has been shown to play an essential role in the regulation of gastrulation movements, the molecular mechanisms of how Wnt signals intracellularly and how it regulates the tissue movements remain elusive. We have shown that Wnt/PCP signaling activates the protein ubiquitination/degradation system, which is essential for cell motility during *Xenopus* gastrulation. In order to clarify how the ubiquitination system is involved in the Wnt/PCP pathway, we focused on β-TrCP (transducin-repeat containing protein), a component of E3 ubiquitin ligase complex. β-TrCP has been shown to regulate the canonical Wnt pathway by ubiquitinating β-catenin, but it is not known whether β-TrCP is involved in the noncanonical Wnt/PCP pathway. Our study revealed that β-TrCP plays an essential role in this signaling pathway. Dishevelled (Dsh) is a cytoplasmic protein, which plays pivotal roles in the canonical and noncanonical Wnt pathways. When the Wnt/PCP pathway is activated, Dsh is translocated to the plasma membrane. β-TrCP colocalized with Dsh both before and after the activation of Wnt/PCP signaling (Figure 2). Furthermore, knockdown of β-TrCP by specific antisense morpholino oligonucleotides inhibited gastrulation

movements and Dsh translocation to the plasma membrane. This translocation is essential for Dsh to activate the downstream signaling pathway, and thus, our results strongly suggest that β-TrCP plays a key role in this signaling pathway. We are currently investigating the regulatory mechanisms of β-TrCP activity and its substrates to regulate gastrulation movements in *Xenopus* embryo.

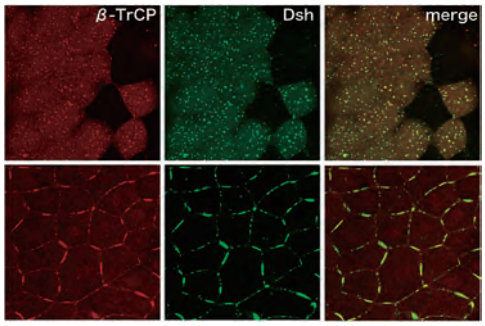

Figure 2. Colocalization of β-TrCP with Dishevelled (Dsh) in ectodermal cells of *Xenopus* embryo. Upper panels; Dsh showed punctate localization (green) and colocalized with β-TrCP (red). Lower panels; When Wnt/PCP signaling was activated, β-TrCP and Dsh were translocated to the plasma membrane.

III. Cellular morphogenesis during neural tube formation

Closure of the neural tube, like the morphogenesis of all epithelial tissues, fundamentally requires the change and maintenance of cell shape. Neural tube closure requires at least two coordinated morphogenetic events, cell elongation and apical constriction. Although cytoskeletal elements are responsible for such changes, how actin filaments and microtubules are regulated in this process *in vivo* is unknown.

We found that neural tube closure in *Xenopus* critically depended on two proteins, MID1, the responsible protein for Opitz G/BBB syndrome (OS) in humans, and MID2. OS is characterized by midline malformations, including hypertelorism, hypospadias, cleft lip/palate, and brain abnormalities. Depletion of the *Xenopus* MIDs disrupted epithelial morphology in the neural plate, leading to neural tube defects. In the MID-depleted neural tube, the normal apical accumulation of the cadherin/β-catenin complex in the cells and formation of the basal lamina were perturbed (Figure 3). Furthermore, the xMID knockdown destabilized and disorganized apicobasally-polarized microtubules in the neural plate, and these abnormalities accounted for the abnormal epithelial cell morphologies and neural tube closure. We also found that the xMIDs and their interacting protein Mig12 were coordinately required for microtubule stabilization during remodeling of the neural plate. Since the depletion of the xMIDs also affected the optic vesicle, cement gland, and kidney, all epithelial organs, we propose that similar mechanisms underlie the normal morphogenesis of epithelial tissues and organs where MIDs are expressed, including the tissues affected in Opitz G/BBB syndrome patients.

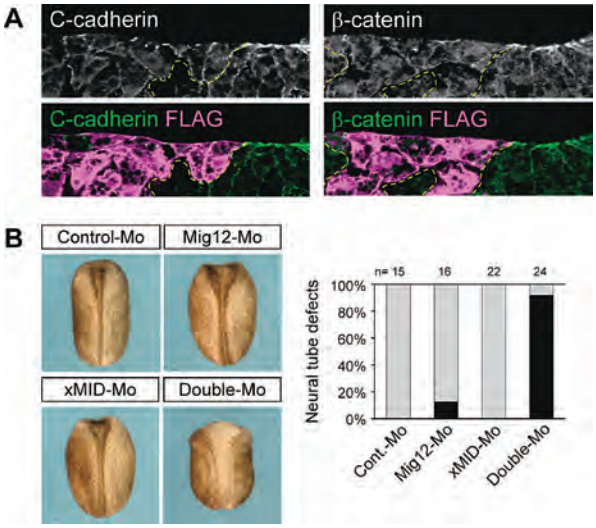

Figure 3. A, Transverse sections of the neural plate containing MID-depleted cells (magenta), stained with antibodies against C-cadherin and β-catenin. B, Functional interaction of Mig12 and MIDs. Mild-knockdown of Mig12 or MIDs by antisense Morpholino (Mo) caused slight delays in neural tube closure. Double-knockdown of Mig12 and MIDs (Double-Mo) induced severe neural tube defects.

We also investigated roles of an adhesion molecule nectin, which belongs to the immunoglobulin-like cell adhesion molecule in the neural tube formation of *Xenopus*. Depletion of nectin-2, one of the nectin family members, from early embryos resulted in incomplete neural fold formation. Cellular analyses revealed less accumulation of F-actin at the apical surface, causing an aberrant apical constriction, a cell-shape change that is required for neural tube folding. Furthermore, we found nectin-2 functionally cooperates with N-cadherin to synergistically enhance apical constriction, highlighting the cooperative action between spatiotemporally upregulated nectin-2 and N-cadherin in the neural plate.

IV. *Brachyury*-downstream gene sets in a chordate, *Ciona intestinalis*

Brachyury plays a pivotal role in the notochord formation in ascidian embryos. *Ciona intestinalis Noto4* (*Ci-Noto4*) was isolated as a gene downstream of *Ci-Bra*. This gene encodes a 307 amino-acid protein with a C-terminal phosphotyrosine interaction domain (PTB/PID). Expression of *Ci-Noto4* commences at the neural plate stage and is specific to notochord cells. Suppression of *Ci-Noto4* levels with specific antisense morpholino oligonucleotides resulted in the formation of two rows of notochord cells owing to a lack of midline intercalation between the bilateral populations of progenitor cells. In contrast, injection of a *Ci-Bra*(promoter):Ci-Noto4-EGFP construct into fertilized eggs disrupted the localization of notochord cells in the

embryonic trunk (Figure 4). Ci-Noto4 overexpression did not affect cellular differentiation in the notochord, muscle, mesenchyme, or nervous system. Analysis of Ci-Noto4 regions that are responsible for its function suggested significant roles for the PTB/PID and a central region, an area with no obvious sequence similarity to other known proteins. Genes involved in planar cell polarity, such as *Ci-prickle*, did not show similar functional effects when compared with *Ci-Noto4*. These results suggested that PTB/PID-containing Ci-Noto4 is essential for midline intercalation of notochord cells in chordate embryos.

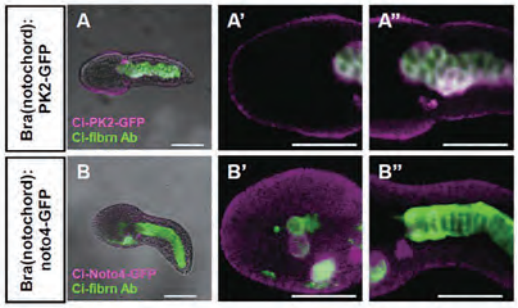

Figure 4. Effects of Ci-prickle and Ci-Noto4 overexpression. (A-A'') A Ci-Bra(promoter):Ci-Prickle-EGFP construct was injected into fertilized eggs. Ci-Prickle-EGFP overexpression did not cause the notochord cells to disperse in the trunk region as was observed in Ci-Noto4 overexpressing embryos. (A-A'', B-B'') Magenta marks Ci-Bra(promoter):Ci-Prickle-EGFP expressing or Ci-Bra(promoter):Ci-Noto4-EGFP expressing notochord cells with anti-GFP antibody. Green marks endogenous Ci-fibrn protein localization with anti-Ci-fibrn antibody. Scale bars = 50μm

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Germ cells are specialized cells that can transmit genetic materials from one generation to the next in sexual reproduction. All of the other cells of the body are somatic cells. This separation of germ and somatic cells is one of the oldest problems in developmental biology. In many animal groups, a specialized portion of egg cytoplasm, or germ plasm, is inherited by the cell lineage which gives rise to germ cells. This cell lineage is called germline. The germline progenitors eventually migrate into the gonads, where they differentiate as germline stem cells (GSC) to form eggs and sperm when the organisms are physically matured. Our laboratory aims to find the molecular mechanisms regulating germline segregation, germline sex determination and GSC niche formation and function in *Drosophila*.

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

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

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

II. Mechanism regulating sex determination of pole cells

Germ cells must develop along distinct male or female paths to produce eggs or sperm. It has been reported that germline sexual identity is regulated by a masculinizing signal from the somatic gonadal cells to pole cells within the embryonic gonads. However, we have found that reduction of sumoylation causes apoptosis of the migrating pole cells in a female-specific manner, suggesting that sexual identity has already been established in the pole cells prior to gonad formation.

Here we show that Sex lethal (Sxl) acts autonomously in the germline to induce female development in Drosophila. Sxl is transiently expressed in the germline progenitors, or pole cells, during their migration to the gonads. Its expression is detected in a female-specific manner and is necessary for feminization of pole cells before they form the gonads. Furthermore, ectopic expression of Sxl in male (XY) pole cells is sufficient to induce female fate in these cells, and the resulting pole cells are able to produce functional eggs within female (XX) soma.

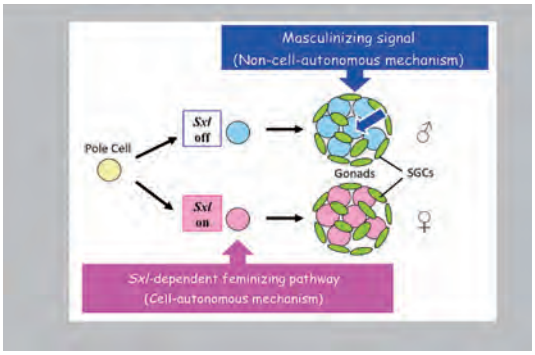

Figure 1. A model for the regulation of sexual dimorphism in pole cells.

The germline sexual identity is regulated both by sex of the surrounding soma and by a cell-autonomous cue. Our findings provide strong evidence that Sxl has a pivotal role in the germline-autonomous mechanism regulating sex determination. XX pole cells initiate female sexual identity based on their Sxl expression, while, lacking Sxl expression in XY pole cells, male sexual fate occurs primarily by a signal from gonadal soma (Figure 1). One remarkable

example of germline-autonomous regulation of sexual dimorphism has been reported in a primitive animal, cnidarian *Hydra*. It has long been known that sex of the germline is not influenced by the surrounding soma, and the germline, rather than soma, determines the phenotypic sex of the polyp. Thus, we speculate that germline-autonomous regulation of sex is a primitive trait conserved throughout the evolution of animals, and somatic control may have evolved with the emergence of mesodermal tissues, including gonadal soma.

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

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

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

We further found that Notch signaling induces hub differentiation. Notch is activated in almost all of the SGCs within male embryonic gonads, suggesting that the posterior SGCs, as well as the anterior SGCs, have the capacity to contribute to hub differentiation. Since hub differentiation is restricted in the anterior SGCs, the posterior SGCs should be repressed to become hub cells. Although Sev acts as a repressor for hub differentiation in the posterior SGCs, expression of a constitutive-active form of Sev is unable to inhibit hub differentiation observed in the anterior SGCs, suggesting that Sev does not have a sufficient ability to repress hub differentiation. Thus we speculate that another RTK signaling pathway has a key role to restrict hub differentiation in the anterior SGCs.

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

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

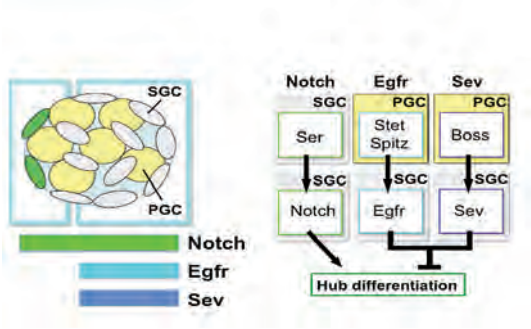

Figure 2. Hub differentiation is controlled by negative regulators, Sev and Egfr and a positive regulator, Notch.

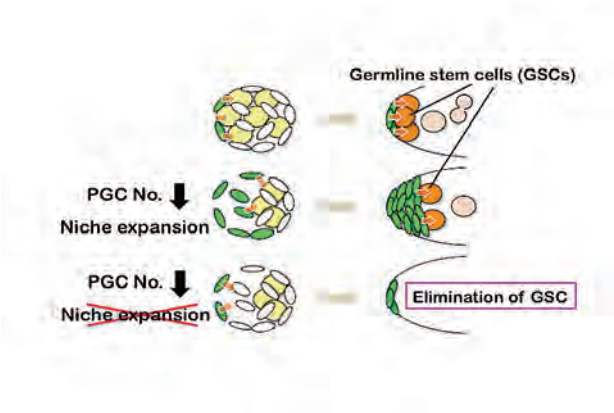

Figure 3. Mechanism securing the germline stem cells. Decrease in PGC number causes niche expansion, thereby recruiting a small number of PGCs as GSCs.

IV. The role of heparan sulfate proteoglycan in the germline-stem-cell niche

Stem cells posses the remarkable capacity to generate daughter cells that retain a stem-cell identity and others that differentiate. Stem cells reside in dedicated cellular microenvironments termed stem-cell niches. These niches dictate stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. *Drosophila* GSCs are one of the most characterized among animal stem cells. Recent studies show that several signaling pathways, such as TGF-beta and JAK/STAT are essential for maintenance of the GSC niche. GSCs are surrounded by somatic gonadal cells, named as niche cells, which form the physical area of the niche and are

responsible for signaling molecule secretion. Despite the importance of these molecules, it is not well understood how these secreted molecules can precisely define the region of the GSC niche.

Heparan Sulfate Proteoglycans (HSPGs) are a group of glycoproteins which are expressed on the cell surface and/or in the extracellular matrix. Recent in vivo studies have shown that HSPGs play critical roles in regulating signaling during development by a variety of mechanisms, including controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally* can control distribution of the TGF-beta ligand, Dpp, and establish a Dpp morphogen gradient during wing development.

We recently identified the *Drosophila* glypicans, *dally* and *dally-like* as important components of the GSC niche in both sexes. *dally* and *dally-like* were strongly expressed in female and male GSC niche cells, respectively. Mutant animals for these glypicans showed significant reduction of GSC number as a result of failure of proper signal activation. Furthermore, ectopic expression of *dally* in female gonads caused an increase in GSC number. Based on these observations, we conclude that glypicans define GSC niche region by regulating signaling pathways involved in GSC maintenance. We present the model that glypicans have a role in defining the GSC niche by controlling ligand stability or distribution in the niche region.

Figure 4. *dally* is an essential component of the GSC niche. (A-C) Anterior most region of ovariole (Germarium) from control (A), *dally* mutant (B) and *dally*-ectopically-expressed animal (C). In control female, two to three GSCs (arrows) exist at the anterior tip of the germarium (A). Once *dally* function is lost, GSC number is decreased, and consequently germarium lacking GSCs are frequently observed (B, bracket). Conversely, germarium in which *dally* is ectopically expressed possess more than ten GSCs (C, bracket). Green signal shows germline marker, Vasa protein, and magenta signal shows membrane-skeletal protein, Hts. GSCs are identified as spherical shaped Vasa positive cells with dot shaped Hts signals.

V. Transcriptional regulation in Malaria parasites (S. Shigenobu)

Gene expression in Plasmodium parasites undergoes significant changes in each developmental stage, but the transcription factors (TFs) regulating these changes have not been identified. We identified a Plasmodium TF (AP2-O) that activates gene expression in ookinetes, the mosquito-invasive form, and has a DNA-binding domain structurally related to that of a plant TF, Apetala2 (AP2). The Plasmodium TF activates a set of genes, including all genes reported to be required for midgut invasion, by binding to specific six-base sequences on the proximal promoter.

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- Hashiyama, K., and Kobayashi, S. (2009). Expression of genes involved in sumoylation in the *Drosophila* germline. Gene Expression Patterns *9*, 50-53.
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The body and tissues of the developing embryo are repeatedly divided into sub-regions specified by characteristic gene expression and morphology. The process that gives rise to these sub-regions, according to a defined pattern, is called "pattern formation" or "patterning." The most popular model to explain the patterning process is the "morphogen gradient and threshold" theory. Many genetic results indicate that secreted signal proteins such as Wnt, BMP, and Hedgehog function as morphogens in many aspects of patterning processes. In spite of the accumulation of genetic evidence, however, the biochemical characteristics of morphogens, including modification and higher order structure, remain to be elucidated. Thus, one of our major goals is to reveal *the real image* of morphogens and the molecular mechanism underlying the formation of morphogen gradients, including the secretion and extracellular transport of these morphogens.

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

I. Secretion and modification of Wnt proteins

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

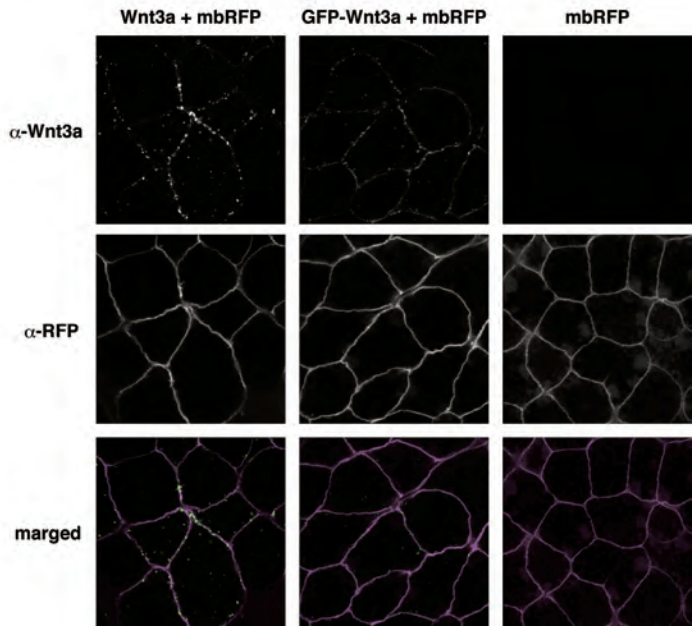

Figure 1. Visualization of Wnt-3a proteins secreted from Xenopus epidermal cells. A GFP-tagged form of Wnt-3a protein, as well as the authentic form, can be observed as punctate signals around the edge of cells. We can use the GFP tagged-Wnt proteins for visualization of extracellular trafficking of Wnt proteins.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

To better understand the molecular mechanism of secretion and gradient formation of Wnt proteins, we are carefully examining the biochemical characteristics of Wnt proteins from cultured cells. We are also trying to reveal the role of palmitoleoylation and the molecular mechanism underlying the extracellular transport of Wnt proteins during embryogenesis using zebrafish and frog embryos (Figure 1).

II. Characteristics of genes required for the development of somite or pharyngeal arches -----The function of mouse Ripply1 and 2 in the rostro-caudal patterning within a somite -----

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, which is a process including inter-somitic transition, a segmental pre-pattern, characterized by segmental gene expression, is established in the anterior PSM. The establishment of the segmental pre-pattern in the anterior PSM has been revealed to require a number of processes regulated by many transcription factors and signaling molecules.

The spatial pattern of somites is characterized not only by the periodical borders between neighboring somites, but also by the rostral and caudal compartments within a somite. These compartments are subsequently segregated and re-fused with adjacent compartments to form vertebra. In addition, the rostro-caudal pattern defines the migration of neural crest cells and motor axons. In contrast to the many patterning processes that have already been revealed, the rostro-caudal patterning of a somite is unique in that a spatial pattern is established with temporal periodicity. However, the precise molecular mechanism by which the rostro-caudal pattern is established remains unclear.

We showed that a gene identified by our *in situ* hybridization screening, *ripply1* is required for this transition. Ripply proteins suppress Tbx-mediated transcription by recruiting the Groucho/TLE co-repressor. Rippy1 and 2 are expressed in the anterior PSM and in several newly formed somites in zebrafish and mouse embryos (Figure 2). In *ripply1*-deficient zebrafish embryos somite boundaries do not form, the characteristic gene expression in the PSM is not properly terminated, and the initially established rostro-caudal patterning in the segmental unit is not maintained, whereas paraxial mesoderm cells become differentiated. Thus, *ripply1* plays a role in the maintenance of the rostro-caudal patterning.

In addition, Notch signaling and Mesp2 activity are also required for the rostro-caudal patterning of a somite. For instance, mouse embryos defective in Notch signaling, caused by knocking out *Dll1* or *Presenilin1*, show rostralized somites, whereas those lacking Mesp2 activity exhibit caudalization of their somites. Consistent with their roles in rostro-caudal patterning, the Notch and Mesp2 active domains become contracted in the caudal half of S0 (the prospective somite in the most anterior PSM) and in the rostral half of S-1 (the prospective somite posterior to S0), respectively. Interestingly, *Mesp2* is required for *Ripply2* expression, indicating that *Mesp2* suppresses its own expression by activating Ripply2 expression. Thus, rostro-caudal patterning appears to be established through molecular interactions between these molecules.

Given that Mesp2 expression is dynamically changed in the anterior PSM in association with the traveling wave of Notch activity, characterization of the dynamism of Mesp2 expression and Notch activity would be important for understanding the mechanism of rostro-caudal patterning. Furthermore, the effect of Ripply on the dynamic movement of Mesp2 expression and Notch activity would be revealed. Therefore, we examined the dynamic processes of this patterning by exhaustive examination of periodical changes in the location of the Notch active domain and the Mesp2 protein domain in wild-type and *Ripply*-deficient mouse embryos at several distinct phases of the segmentation cycle. We examined the spatial movement of Notch activity with Mesp2 protein localization in wild-type embryos and those

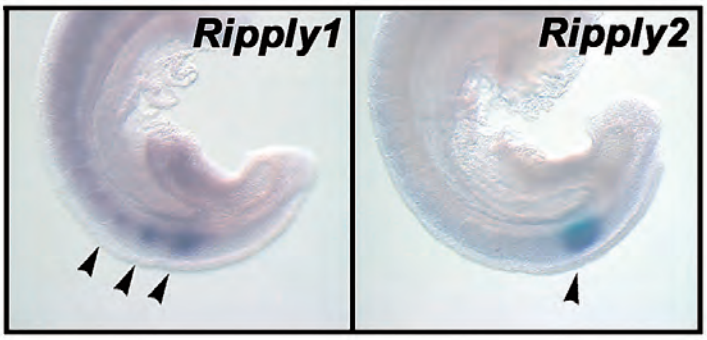

Figure 2. Expression patterns of mouse Ripply1 and 2 in somite development. Both Ripply1 and 2 are segmentally expressed in the anterior presomitic mesoderm.

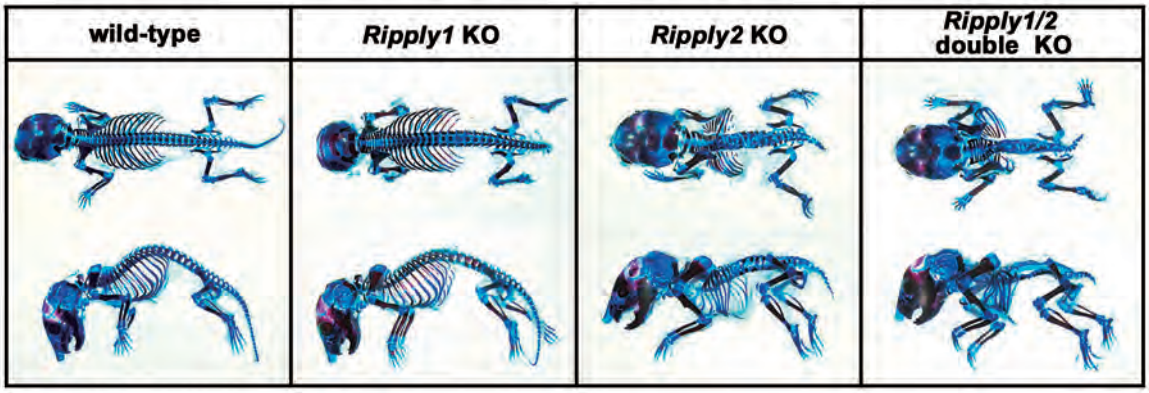

Figure 3 Segmental abnormalities observed in Ripply1 and 2 double knockout embryos. Segmental defects of somites appear in structures of vertebrae and ribs. Ripply1 deficiency enhances rostralized phenotype of Ripply2 knock-out embryos in somite development. .

defective in the two *Ripply* genes expressed in the PSM.

Mesp2 protein appears first as a thin band in the traveling Notch domain. In wild-type embryos, the Mesp2 band expands anteriorly to the expression front of Tbx6, an activator of *Mesp2* transcription. Notch activity becomes localized further anteriorly to this Mesp2 domain, but does not pass over the anterior Mesp2 domain generated in the previous segmentation cycle. In *Ripply1/2*-deficient embryos, the Mesp2 band becomes more expanded and the Notch domain is finally diminished. Interestingly, *Ripply1/2*-deficient embryos exhibit anterior expansion of Tbx6 protein domain, suggesting that Ripply1/2 regulates *Mesp2* expression through Tbx6 degradation. We propose that the rostro-caudal pattern is established by dynamic interaction of Notch activity with two Mesp2 domains, which are defined in successive segmentation cycles by Notch, Tbx6 and Ripply1/2.

In addition to these roles of Ripply1 and 2 in somite segmentation, we have also examined the role of another Ripply gene in the development of pharyngeal arches.

Publication List

〔Original paper〕

- Agalliu, D., Takada, S., Agalliu, I., McMahon, A.P., and Jessell, T.M. (2009). Motor neurons with axial muscle projections specified by Wnt4/5 signaling. Neuron *61,* 708-720.

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.

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

I. Cell differentiation in pre-implantation development

During the 4 days after fertilization, the mouse embryo proceeds in its development within the zona-pellucida, an extra-embryonic membrane surrounding the embryo proper, and reaches the blastocyst stage. In the mouse blastocyst, two types of cells, namely cells of the inner cell mass (ICM) and cells of the trophectoderm (TE) can be distinguished. This is the initial cell differentiation during mouse development. Oct4 (Oct3/4) and Cdx2 are differentially expressed in the ICM and TE respectively, and it is suggested that Oct4 functions as a factor to maintain pluripotency of ICM cells. The onset of the specification of these two cell types has not been defined. The initial stage when Oct4 and Cdx2 are differentially expressed is known to be early in the morula stage. The cells located at the outside express Cdx2, suggesting that specification has occurred in these outside cells. It is hypothesized that the cells located inside the morula mainly contribute to the ICM and cells facing the embryonic surface will differentiate into TE cells. However, this inside-outside hypothesis is based on the analysis of separated blastomeres, and continuous information from whole embryos was very limited. We therefore analyzed division orders of cells and expression profiles of differentiation markers relating to the position of cells within embryos to reveal the different characteristics of each blastomere in the embryo.

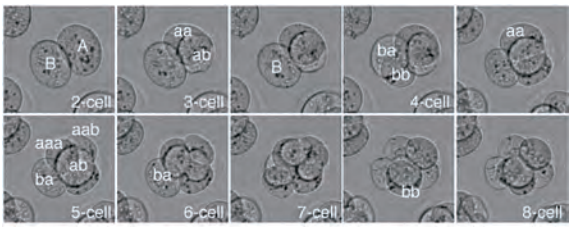

Figure 1. Analysis of division patterns until the 8-cell stage. Blastomere A divided earlier than blastomere B to give rise to the 3-cell stage. The four blastomeres aa, ab, ba, bb divided to reach 5, 6, 7, and 8-cell stages, respectively.

The dividing patterns of blastomeres until the late 8-cell stage were analyzed using bright field time-lapse recording images. The time point of each blastomere division was recorded, and the division patterns were analyzed. All six possible types of division pattern were observed. Of all cases, the type where all the daughter cells of the early dividing cell precede in the next pattern was most common. When the period of the 3-cell stage was examined, there was correlation with the appearance of different patterns and the length of the stage, which showed a wide variety lasting up to 220min. The most common pattern was seen in embryos with a longer 3-cell stage period, and variant patterns occurred more often during short 3-cell stage periods. This suggests that the division order might be altered when the divisions of blastomeres occurs synchronously. We did not observe any relationship between the division order and future cell fates.

We analyzed the positioning of cells within each embryo using optical section images of phalloidin and Hoechst staining obtained by confocal microscopy. We defined an outer cell as a cell facing the embryonic surface of the

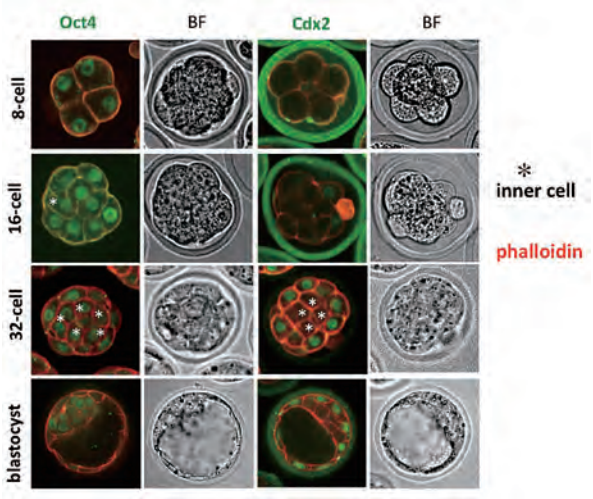

Figure 2. Localization of Oct 4 and Cdx2 protein during preimplantation mouse development. Cell shape was visualized by phalloidin staining.

embryo and an inner cell as a cell not facing the surface of the embryo but surrounded by other cells. At the 8-cell stage, even after compaction, almost all the cells were outer cells. At the 16-cell stage, inner cells were observed. The average number of inner cells was 1.7. All the embryos contained inner cells at the 32-cell stage. Thus, the geographical differences between the inner and outer cells can be initiated later than the 8-cell stage. The Oct4 proteins staining signals were observed in all embryos both in inner and outer cells until the 32-cell stage. The Cdx2 staining signals were different from that of Oct4. Cdx2 was initially observed in a few cells of the 8-cell stage, and the numbers of Cdx2-positive cells increased as development progressed. Cdx2 signals were observed in most of the outer cells at the 32-cell stage. Thus Cdx2 and Oct4 were co-expressed in most outer cells at the 32-cell stage. These results suggest that the differentiation of the two cell types might correlate with the position of the cells within the embryo.

We have started to prepare transgenic mice where fluorescent protein expressions are driven by the gene regulatory elements of genes involved in cell differentiation. These will be used for live imaging of gene expression during cell differentiation.

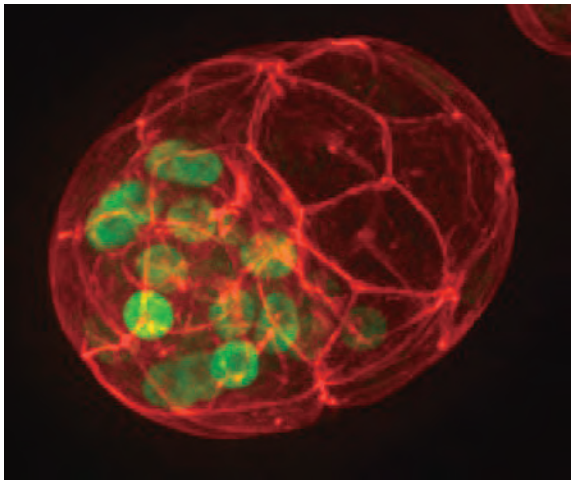

Figure 3. Localization of Nanog protein at mid blastocyst stage. This protein is one of the candidates applied for live cell imaging.

II. Planar cell polarity in mouse oviduct

Planar Cell Polarity (PCP) is the asymmetric organization within the epithelial cells along the plane of the epithelium in tissues. We are focusing on the epithelial cells of the mouse oviduct. The multiple cilia on the cell surface beat back and forth along the axis from the ovary to the uterus, and this beating must be directionally controlled to transport eggs to the uterus. Thus, these ciliated cells may have clear PCP from ovary to uterus. Our aim is to reveal the molecular and cellular mechanisms of regulating PCP in the mouse oviduct.

We observed the movement of oviduct epithelial cilia by high-speed CCD camera, and confirmed that the cilia generally beat along the axis from ovary to uterus. To understand how these movements of cilia can make a flow of fluid to transport follicles from the ovary to the uterus, we

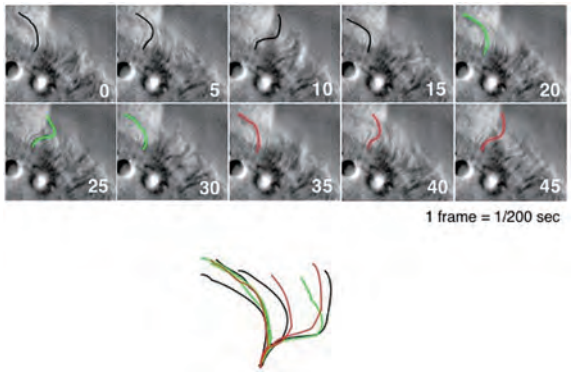

Figure 4. Observation of movements of the multiple cilia of the oviduct epithelial cells. The numbers shown in bottom indicate frame numbers of the movie. The shape of a cilium is traced on the snap shot images taken by high speed CCD camera. The movements of the cilium are represented on the bottom.

carefully analyzed the movements of cilium. There was a wide variety of frequency in the beating of cilia. These cilia did not necessarily makea coordinated synchronous beating. The effective stroke (forward movement along the ovary-uterus axis) of cilia is 2-3 folds faster than the recovery stroke (backward movement). This differential speed of each stroke might provide a force to generate the functional flow. We are studying how these ciliated cells generate coordinated and functional flow in the oviduct. To analyze the beating in more detail, we are planning to develop a tool to automatically analyze cilia beat frequency at multiple points simultaneously. We are also preparing experiments to uncover molecular mechanisms underlying PCP formation in the oviducts, mainly by focusing on the involvement of classical PCP genes and their products.

Publication List

〔Original papers〕

- Fujimori, T., Kurotaki, Y., Komatsu, K., and Nabeshima, Y. (2009). Morphological organization of the mouse preimplantation embryo. Reprod Sci. *16*, 171-177.
- Yuri, S., Fujimura, S., Nimura, K., Takeda, N., Toyooka, Y., Fujimura, Y., Aburatani, H., Ura, K., Koseki, H., Niwa, H., Nishinakamura, R. (2009). Sall4 is essential for stabilization, but not for pluripotency, of embryonic stem cells by repressing aberrant trophectoderm gene expression. Stem Cells *27*, 796-805.

DIVISION OF GERM CELL BIOLOGY

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Mammalian spermatogenesis represents a highly potent and robust stem cell system. Decades of research, including detailed morphological examinations, post-transplantation repopulation, and *in vitro* culture, have made it one of the most intensively studied mammalian tissue stem cell systems. However, the nature of the stem cells and their control, as well as their niche, remains largely unknown. The Division of Germ Cell Biology aims to fully understand the mammalian spermatogenic stem cell system, mainly using mice. In 2009, our second year at NIBB, we have tackled a number of important issues in mouse spermatogenic stem cells.

I. Background: The mammalian spermatogenesis

Mammalian testes continually produce numerous sperm during the reproduction period. Lines of investigations that emerged in the 1950s and involve detailed morphological analyses established the backbone of mammalian spermatogenesis research. The morphologically most primitive spermatogonia in the adult mouse testis are A_s or A_{single} spermatogonia (single, isolated spermatogonia). Their progeny remain interconnected due to incomplete cytokinesis, forming syncytial cysts of 2^n cells (2, 4, 8, 16 etc.). It has been experimentally established that "undifferentiated spermatogonia" (or "A_{undiff}" hereafter), which contribute <1% of the entire testicular cells and consist of A_s, A_{pr} (A_{paired}; interconnected two-cell cysts), and A_{al} ($A_{aligned}$; cysts of 4, 8, 16 or occasionally 32 cells) contain stem cells.

Then, which subfraction of A_{undiff} consists of the actually self-renewing stem cell compartment in homeostasis and how do they behave (proliferate, self-renew or die) in the testis? The prevailing, so-called "A_s model", which was originally proposed in 1971, proposes that A_s is the only cell type that can act as stem cells, while the interconnected population of A_{undiff} (A_{pr} and A_{al}) is devoid of stem cell capacity.

II. Toward the nature of the mouse spermatogenic stem cell system that includes reversibility

Despite that the "A_s model" is comprehensive and persuasive, this model is not based on the direct analyses of the cells' behavior. Thus, the A_s model warrants functional evaluations.

Similarly, corollaries of this model are that all the A_s cells are functionally equivalent and uniformly act as the stem cells, and that this defined population of 'stem cells' plays an active role in every aspect of the stem cell functions, i.e., maintenance of homeostasis, post-transplantation colony formation, and post-injury tissue regeneration.

We have previously shown that no single stem cell population acts in every aspect of stem cell function: Cells that support the steady-state spermatogenesis are different from those that support colony-formation and/or regeneration (Nakagawa et al., Dev. Cell, 2007). Based on this finding, we propose the functional hierarchy in the spermatogenic stem cell compartments as shown in Figure. 1. We propose that in addition to the actually self-renewing population (actual stem cells), an extended population of cells that retain the self-renewing potential but are destined for differentiation (potential stem cells) exist, which plays an important role for the continuity and robustness of the stem cell system. In case of actual stem cell loss, potential stem cells might revert to the self-renewing mode and replenish the actual stem cell pool (Figure 1).

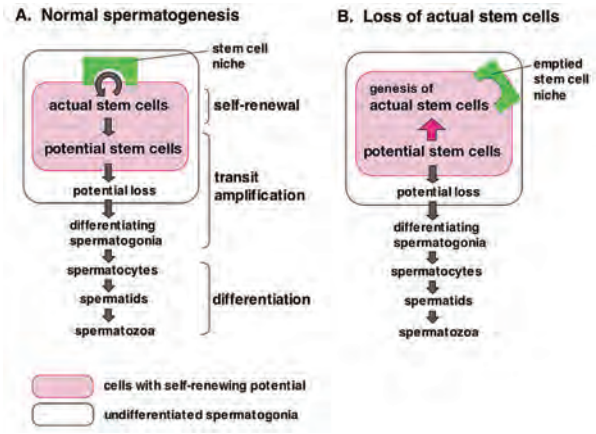

Figure 1. Proposed model of the functional compartments in mouse spermatogenesis (Nakagawa et al., 2007).

In 2009, by revisiting the A_s model, we investigated the cell population of A_{undiff}. For this attempt, we introduced a gene expression profile for describing the heterogeneity of the cells, in addition to the number of chained cells or the length of the cysts, on which the "A_s model" entirely depended on. Several studies, including ours, have been establishing that the populations of cysts that compose the same number of spermatogonia, which are classically considered to be homogeneous, are indeed heterogeneous in terms of patterns of gene expression. Therefore, a new scope has been raised that the entire A_{undiff} population is more heterogeneous than has previously been considered.

We have also investigated the behaviors of these A_{undiff} subpopulations in homeostasis (steady-state spermatogenesis) and during regeneration after tissue insult. We revealed that a particular subset of A_{undiff} changes their behavior dynamically between homeostasis and regeneration: In steady-state, they differentiate without self-renewal, but in

regeneration, they are willing to get back into a self-renewing stem cell state. This finding provides a novel view for stem cell biology and explains why the stem cells can change the behavior between homeostasis and regeneration so smoothly. In homeostasis, the number of stem cells should be constant, while in regeneration, they increases to recover the stem cell pool quickly. This feature is crucial for the continuity of tissue functions.

III. Investigating the temporal control of differentiation and establishment of the testicular tissue

In seminiferous tubules, spermatogenic differentiation occurs in a periodical manner, which results in the formation of beautiful stratification of different steps of differentiating cells within the seminiferous tubules (Figure 2A). Essentially, this is caused by the periodical differentiation of A_{undiff} with a regular interval of 8.6 days (white arrows in Figure 2B).

We have investigated how this periodical event is controlled. This question can be generalized to how the timing of A_{undiff} differentiation is regulated. We paid special attention to the involvement of Sertoli cells and the role of retinoic acid (RA) signaling. The importance of RA has been recognized for decades based on the finding that deficiency in vitamin A (VA), the dietary precursor of RA, causes a severe spermatogenesis defect in which differentiation of A_{undiff} is affected, and differentiating cell types are all lost.

We have revisited this VA-deficient (VAD) model and revealed the central role of RA not only in the control of the timing of A_{undiff} differentiation, but also in the regulation of the functional change of the supporting somatic Sertoli cells, which are huge cells that nourish the stratified germ cells. The local retinoid metabolism in the seminiferous tubules involves multiple cell types of germ and somatic cells. Therefore, co-operation between different cell types that are located adjacently may control the differentiation of A_{undiff}.

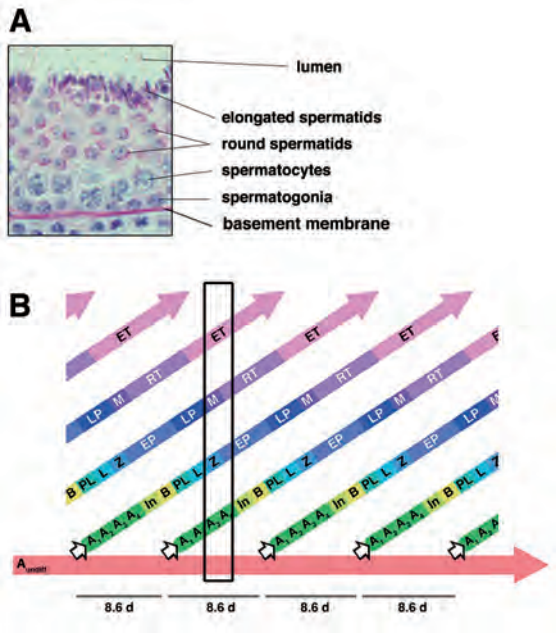

Figure 2. Stratified tissue architecture in mouse seminiferous tubules.

(A) Appearance of a representative part of the seminiferous tubules. Note the beautifully stratified germ cells at different steps of differentiation.

(B) A scheme that explains the formation of the stratified architecture shown in (A). The population of A_{undiff} (red) persists for a long period and gives rise to A1 differentiating spermatogonia periodically with a regular interval of 8.6 days. As a result, a regular stratification of the differentiating germ cells is formed, as indicated by the rectangle.

IV. Investigating the nature of the stem cell niche

Evidence suggests an intimate relationship between stem cells and the niche microenvironment in seminiferous tubules. It is difficult to identify the nature and function of the niche, however, because seminiferous tubules do not exhibit suspicious sub-structures. Moreover, actual stem cells can be identified only functionally, and their histological detection has not yet been achieved. Therefore, our current aim has been to clarify the niche of A_{undiff}.

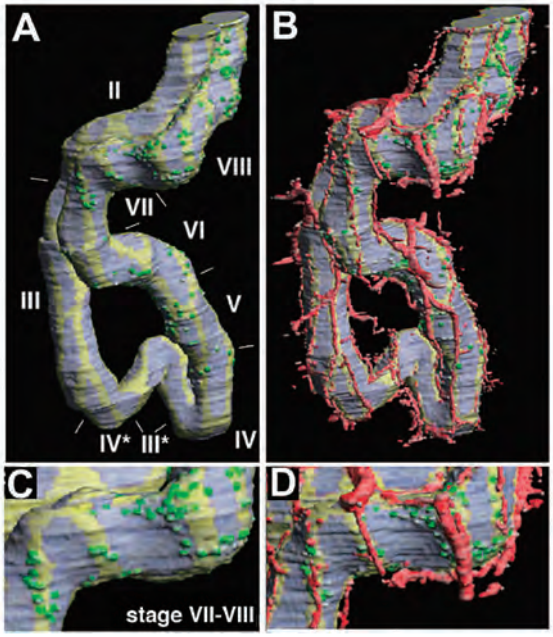

Figure 3. Localization of A_{undiff} revealed by three-dimensional reconstruction (Yoshida et al., Science, 2007)

Computationally reconstituted three-dimensional images of the seminiferous tubules based on 280 serial sections. A_{undiff} (green) show biased localization to the blood vessel network (red) and the area adjacent to the interstitium (yellow). (A, C) and (B, D), without or with blood vessels, respectively. Roman numerals indicate the stage of the seminiferous epithelium.

The seminiferous tubules exhibit a convoluted tubular structure with a diameter of ~200μm: Individual tubules connect to the common outlet of the mature sperm (rete testes) with both ends and form loops. Spermatogenesis occurs evenly throughout the inner surface of the tubules. Therefore, in mouse testis, an overall 'polarity' that covers the entire organ cannot be recognized, making a good contrast to the *Drosophila melanogaster* germline stem cell system.

By taking advantages of live-imaging and three-dimensional reconstruction, we previously revealed that A_{undiff} preferentially localizes to the limited regions of seminiferous tubules that are adjacent to blood vessels and interstitial cells that surround the tubules (Figure. 3). In addition, the dynamic migration of spermatogonia from the vasculature proximity to spread throughout the tubules was also observed upon differentiation of A_{undiff} (Yoshida et al., Science, 2007). Based on these findings we proposed that this area may act as the niche for A_{undiff} (Figure 4). We are tackling the cellular and molecular identification of this vasculature-associated niche.

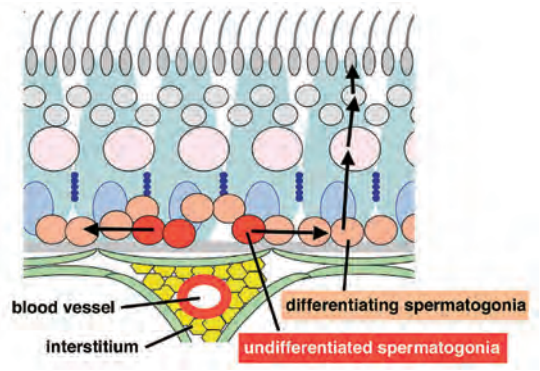

Figure 4. A proposed niche microenvironment for A_{undiff}.

Although the seminiferous tubule is a repeat of the same structure, the proximity to the surrounding blood vessels and interstitial cells specializes the particular region of the basal compartment of the tubules, so that it can act as the niche for undifferentiated A_{undiff} population.

Publication List

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- Uemura, M., Hara, K., Shitara, H., Ishii, R., Tsunekawa, N., Miura, Y., Kurohmaru, M., Taya, C., Yonekawa, H., Kanai-Azuma, M., and Kanai, Y. (2009). Expression and function of mouse Sox17 gene in the specification of gallbladder/bile-duct progenitors during early foregut morphogenesis. Biochem. Biophys. Res. Commun. 2009 Nov. 12

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- Yoshida, S. (2009). Casting back to stem cells. Nat. Cell Biol. *11*, 118-120.

DIVISION OF REPRODUCTIVE BIOLOGY

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

I. Molecular mechanisms of sex determination and gonadal sex differentiation

We identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka (*Oryzias latipes*), the first one in non-mammalian vertebrates. Recently, we have developed a simple, cost effective and gene-specific transgenic RNAi technology for understanding the roles of the zygotic gene products in medaka. Knockdown of DMY in XY gonads resulted in a complete male-to-female sex-reversal in medaka (Figure 1). Importantly, we were able to continue a trans-generational knockdown effect of DMY until at least the F2 generation. Since the RNAi effect is long lasting and inheritable, this will provide a powerful tool for the analysis of not only embryos, but also phenotypic consequences that develop over longer periods of time.

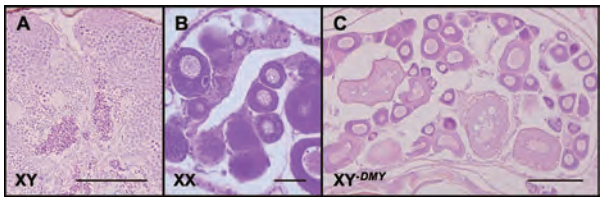

Figure 1. Knockdown of DMY in XY gonads leads to a complete male-to-female sex reversal (C). Gonads from an XY male (A) and an XX female (B). H&E staining was performed on gonads from 50 days after hatching. Bars indicate 50μm.

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

The molecular control of ovarian development in medaka is less understood. We examined whether R-Spondin 1 (RSPO-1), a novel regulator of the Wnt/β-catenin signaling pathway, was involved in ovarian differentiation in medaka. *RSPO-1* is expressed in XX gonads from as early as 0 day after hatching to the adult stage, while the expression was barely detected in XY gonads. Knockdown of RSPO-1 in XX gonads induced female-to-male sex-reversal, while overexpression of RSPO-1 in XY gonads induced male-to-female sex-reversal. Both loss and gain of function evidence indicates that RSPO-1 is critical to initiate the ovary pathway in medaka.

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

II. Molecular mechanisms of sex change

The sex-changing fish *Trimma okinawae* can change its sex back and forth from male to female and then back to male serially, dependent on social status in the harem. The gonad corresponding to the sexual status of the fish remains functional while the other is regressed. The expression of gonadotropin receptors (*GtHR*) was found to be confined to the active gonad of the corresponding sexual phase. The swapping of the gonads is initiated through a switching in the expression of the GtHR, *FSHR* and *LHR*. Changing of the gonads starts with switching of *GtHR* expression discernible within 8-12 h of the visual cue. These two GtHR genes act as mediators to convey the information about the change in social status to the to-be-active gonad.

III. Sexual plasticity in the adult gonochoristic fish

With the exception of certain hermaphroditic species, most vertebrates are thought to lose sexual plasticity after the differentiation of separate gonads/sexes with a single, distinct

gamete type (gonochorism). We treated females of two species of teleost, the Nile tilapia and medaka, with aromatase inhibitors (AI) for up to five months to block the conversion of androgens to estrogens in order to investigate whether sexual plasticity is retained in gonochoristic fish. In both species, suppression of estradiol-17β production via AI treatment caused a rapid degeneration of ovarian tissues, leading to the differentiation and development of testicular tissues. The reduced expression of aromatase (*P450c19a*) with a rise in the expression of *GSDF*, indicates the differentiation of testicular-type somatic cells in the AI-treated gonads. Sex-changed fish show a typical male pattern of estrogen and androgen levels, secondary sex characteristics, producing fertile sperm in the newly formed testes. Our results indicate that gonochoristic fish maintain their sexual plasticity to adulthood and that estrogens play a critical role in maintaining the female phenotype.

IV. Endocrine regulation of oocyte maturation and ovulation

A period of oocyte growth is followed by a process called oocyte maturation and is a prerequisite for successful fertilization. Our studies using vertebrate (fish) and invertebrate (starfish) models have revealed that the basic mechanisms involved in oocyte maturation are the same in these two species despite the differing chemical nature of the hormonal agents involved. In both species, three major mediators have been shown to be involved (*Three step model*): a gonad-stimulating substance (GSS), 1-methyladenine (maturation-inducing hormone, MIH), and a maturation-promoting factor (MPF) in starfish, and gonadotropin (LH), 17α, 20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) (MIH), and MPF in fish.

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

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Laboratory of Molecular Genetics for Reproduction

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Laboratory scope

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

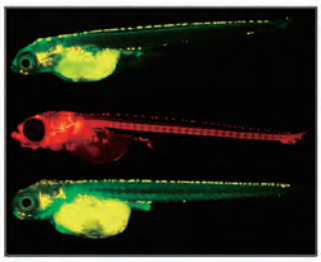

Figure 1. Various transgenic medaka.

I. Canalization of germ cells to feminization vs. male predisposition of somatic cells

We have been analyzing the role of germ cells during the course of sex differentiation of the gonads and have revealed an essential function of germ cells on the development of ovaries.

In wild type medaka, sex is determined genetically in an XY manner. Without germ cells, however, both genetically female and male medaka exhibit male secondary sex characteristics and gonadal somatic cells are masculinized in terms of male-specific gene expression and production of a male steroid hormone. This can be explained by the hypothesis that the female character of germ cells antagonizes the autonomous masculinization of gonadal somatic cells and that balancing between germ cells and gonadal somatic cells is essential for both sex differentiation and maintenance of sex.

In support of this claim, medaka mutants that display a germ cell-hypertrophic phenotype, *hotei*, exhibit female secondary sex characteristics in approximately half of genetic male *hotei* mutants. In the germ cell-deficient *hotei* mutants, none of them show any sex reversal of secondary sex characteristics. In addition, female-specific gene expression seen in genetic male *hotei* mutants is also abolished in the germ cell-depleted mutants. These results indicate that feminization of the gonad in the mutant is a consequence of hypertrophic germ cells but not of a gonadal somatic cell-autonomous event.

The gene responsible for this phenotype is the type II receptor gene for anti-Mullerian homone (*amhrII*). We found that both *amhrII* and its ligand, *amh*, are expressed in gonadal somatic cells (supporting cells) but not in germ cells, indicating that Amh signaling acts autonomously of supporting cells.

II. Amh signaling is involved in sex differentiation by modulating reciprocal interaction between germ cells and somatic cells

The results from both germ cell-deficient medaka and germ cell-hypertrophic mutants (*hotei*) suggest an intrinsic mechanism of sex differentiation that is independent of genetic sex determination. We propose that, irrespective of genetic sex, germ cells have an intrinsic character that canalizes feminization of the gonad (*canalization to female*) while gonadal somatic cells are predisposed to male development (*male predisposition*). According to this proposal, the possible function of the medaka testis determination gene on the Y chromosome is enhancement of masculinization that conquers canalization towards ovary by germ cells. The close interaction has to be present, thereby reciprocal signals between germ cells and somatic cells acting to form proper sex differentiation. Since the complete defect of Amh signaling results in improper sex differentiation, Amh signaling is very likely to modulate the reciprocal signals and balance the two intrinsic signals.

The balancing of the two opposing characters may be a conserved cellular interaction among vertebrates. In fact, there have been several reports of the masculinization of mice ovaries following the depletion of germ cells by genetic or physical manipulations. Even in an undifferentiated gonocholist, zebrafish, which firstly develops ovaries before some of the population turn into males, prior to the development of testis the germ cell number decreases by apoptosis. The suppression of the germ cell number at the early stage of medaka gonadal sex differentiation and the decrease in germ cells in zebrafish can be viewed as different ways of achieving the same result (Tanaka et al., 2008 DGD: Saito et al., Sex. Dev. 2009).

III. Estrogen-producing cells – the earliest female-specific somatic cells develop in a germ cell-dependent manner

As mentioned above, germ cells and the surrounding somatic cells (supporting cells) are important players in the gonads. But there are many other functionally unknown somatic cells in the gonads. Transgenic medaka with GFP fluorescence are powerful tools to analyze them because they provide fine structure and organization of the gonad at single cell levels. We have generated transgenic medaka that enable us to keep track of *cyp19a1* (*aromtatase*)-expressing cells in

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

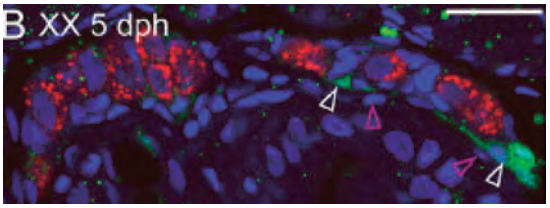

Figure 2. The *cyp19a1*-expressing theca cells (green) are one of the earliest female-specific cells in the female developing gonad. blue: nuclea, red: germ cells. Picture from Nakamura et al (2009).

the gonad by the established method of using medaka BAC (bacterial artificial chromosome) (Nakamura et al., 2008 DGD). The *cyp19a1*-GFP transgenic medaka mimics endogenous *cyp19a1* expression by GFP fluorescence (Nakamura et al., 2009).

Once germ cells enter meiosis, they form the follicular structure with both inner and outer somatic cells surrounding oocytes. The aromatase (product of the gene, *cyp19a1*), which catalyzes a precursor steroid, testosterone, to produce female-specific hormone, estrogen, has generally been used as a good marker of the inner somatic cells called granulosa cells.

The expression of *cyp19a1* in medaka has been observed as early as 5 days post hatching (5dph) in female developing gonads (Figure 2) but not male developing gonads. The *cyp19a1*-expressing cells first appear in the most ventral side of a stromal region adjacent to the epithelium of the female gonad. Immediate after appearance of the *cyp19a1*-expressing cells they were found on the outer layer of follicles but not on the inner layer. This result indicates that the *cyp19a1*-expressing cells are not the granulose cells but theca cells. Very interestingly, another theca cell marker, *P450c17*, is exclusive to *cyp19a1* expression, demonstrating that there are two different types of theca cells on the follicles. We often observe that *cyp19a1*-expressing cells on the ventral stromal region extend their cellular process towards follicles. This observation suggests that the *cyp19a1*-expressing cells in the ventral region are the precursors of one of the theca cells (Figure 3).

Consistent with our result, in the absence of germ cells, female-specific *cyp19a1*-expressing cells are not maintained in the developing ovary (Nakamura et al 2009).

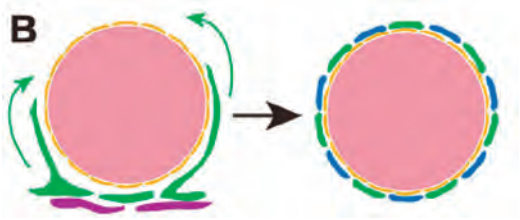

Figure 3. Development of *cyp19a1*-expressing theca cells (green). These cells are different from another type of theca cell expressing *P450c17* (blue). The *cyp19a1*-expressing theca cells first arise near the epithelium (purple) and surround oocytes (pink) cells. Illustration from Nakamura et al (2009)

IV. Expression and syntenic analysis of medaka *nanos* gene family

Nanos is known to be essential for germ cell development. Availability of medaka genomic information allows us to successfully identify four different *nanos* genes on the medaka genome (*nanos 1a, 1b*, *2,* and *3*). *Nanos3* is most conserved among vertebrates at the levels of amino acid sequence and synteny. *Nanos1a* and *1b* are expressed in neuronal cells in common, *nanos2* in gonial cells and *nanos3* specifically in germ cell-lineage (Aoki et al., 2009)

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LABORATORY OF PLANT ORGAN DEVELOPMENT

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

I. Genetic approach

Recent studies of *Arabidopsis* mutants show several genes are involved in the axes-dependent control of lateral organ development. The adaxial-abaxial boundary in the leaf primordium is determined by the precise expression of the adaxial marker gene, *PHABULOSA* (*PHB*), and the abaxial marker genes, *FILAMENTOUS FLOWER* (*FIL*) and *YABBY3* (*YAB3*). We showed that PHB is expressed in cells of the adaxial side and separated clearly from the abaxial side-specific *FIL* gene expressing cells, by the action of microRNA165/166 which targeted the *PHB*, *REV*, and *PHV* messenger RNAs. We also revealed by laser microdissection that specific expression of *MIR165/166* genes in the abaxial side is important for adaxial-abaxial boundary formation.

To examine the mechanisms of boundary formation, we isolated novel mutants which show altered patterns of *FIL* promoter::*GFP* expression, and named them *enlarged fil-expression domain* (*enf*). One of them, *enf1*, forms leaves with enlarged and reduced *FIL*-expression domains. In the extreme cases, leaves are filamentous. This phenotype indicates that *ENF1* is involved in the fixation or maintenance of the position of the adaxial-abaxial boundary. The structure of the SAM is often aberrant in *enf1* mutants. We revealed that the *ENF1* gene encodes an enzyme associated with primary metabolism, and that *ENF1* is strongly expressed in leaf primordia although its expression was not found in the SAM. This indicates that *ENF1* affects the axis-dependent cell fate in leaf primordia. In contrast, another mutant, *enf2*, has leaves with an enlarged *FIL*-expression domain, and the *ENF2* gene encoded a plastid-localized unknown protein. Chloroplast development was repressed in a severe allele of the *enf2* mutant, suggesting that chloroplast development is required for normal differentiation of leaf tissues.

There are methylated tandem repeat sequences in the promoter region of another abaxial side-marker gene, *YAB3*. We revealed that the methylation of the region occurred in a Pol IV/Pol V-dependent manner. We also obtained results suggesting the methylated sequences occur at a higher frequency in the cells of the adaxial-side of leaves than in those of the abaxial side.

A line of unique oblong cells is found at the marginal edge of leaves (Fig. 1 left). We noticed that a homeobox-related gene, *PRESSED FLOWER* (*PRS*) and its homolog, *WOX1*, are required for forming the margin-specific cells. *prs wox1* double mutants completely lack the margin-specific cells in leaves, and interestingly, the abaxial side-specific epidermal cells, which are smaller than the adaxial side-specific epidermal cells, "invade" the adaxial side surface (Fig. 1 right). The results indicate that the margin-specific cells act as a physical barrier separating the epidermal cells of the adaxial-side surface from those of the abaxial-side surface.

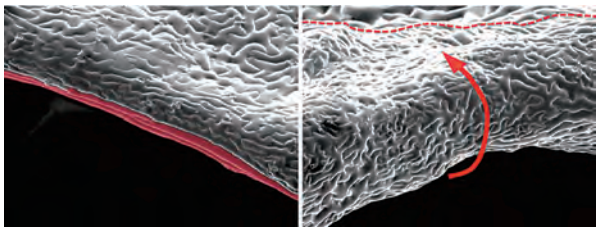

Figure. 1 Oblong cells located at the marginal edge of wild type *Arabidopsis* leaves (painted in red in left panel). In the *prs wox1*double mutant, the long margin-specific cells are absent, and the abaxial side-specific epidermal cells seem to "invade" the adaxial side surface (shown by the arrow in right panel). A red-dashed line indicates the border between the adaxial-side epidermal cells and those of the abaxial side.

II. Biochemical approach

We are taking another approach to study the intercellular signaling system by analyzing small peptides as candidates for intercellular signaling ligands, which are present in the apoplastic region of the SAM. Small peptides collected from apoplast fractions of the heads of cauliflower (*Brassica oleracea* L. var. *botrytis*) and Arabidopsis *apetala1 cauliflower* double mutants were analyzed by peptide

sequence methods or LC-MS/MS methods. We chose about 30 peptides as the candidates, and prepared synthetic peptides based on the obtained sequences. When we applied the peptides to Arabidopsis seedlings, several peptides caused morphological defects in the SAM, vascular tissue and root development. We are currently examining the mechanisms involved.

III. Microsurgical approach

We are also carrying out microsurgical approaches using novel laser-ablating microscopy to investigate the cell-to-cell signaling system working during leaf development. When we ablated a small number of cells at the peripheral of the SAM of young *Arabidopsis* seedlings a few days after germination, some of the newly generated rosette leaves changed to a filamentous structure lacking the adaxial-abaxial identity. This suggests a flow of signal(s) from the SAM to the leaf primordia. Currently, we are ablating some of the margin-specific cells of leaf primordia to analyze the role of these cells.

IV. One-cell gene induction approach

As a new tool for examining the intercellular communication system, we are developing the one-cell induction system *in planta* using the InfraRed Laser Evoked Gene Operator (IR-LEGO) system. We irradiated root epidermal cells of a transgenic line carrying the Arabidopsis heart-shock promoter *hsp18.2*::*GUS* fusion gene. GUS expression was observed in irradiated cells (Fig. 2 left). Induction of a single cell is possible by focusing the irradiation (Fig. 2 right).

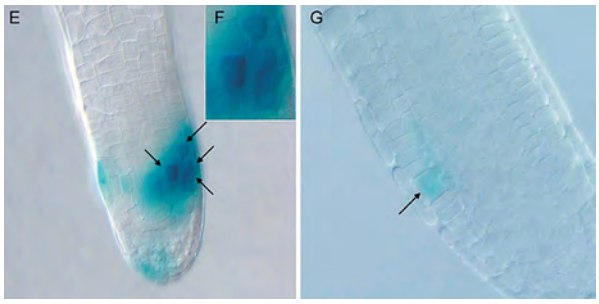

Figure. 2 GUS expression induced by IR laser irradiation to 4 target cells arranged in a diamond shape. The four irradiated cells (arrows) expressed GUS strongly (left). However, weak staining was found in the surrounding cells. Top right panel shows magnified image. GUS expression was restricted to the targeted single cell (arrow in Right) using different laser irradiation settings.

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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 sensation, emotion, behavior, learning, and memory.

I. Mechanisms for retinal patterning during development

Topographic maps are a fundamental feature of neural networks in the nervous system. The retinotectal projection of lower vertebrates including birds has been used as a readily accessible model system of the topographic projection. We have been studying the mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal projection.

Two winged-helix transcription factors, *FoxG1* (previously called *chick brain factor1*, *CBF1*) and *FoxD1* (*chick brain factor2*, *CBF2*), are expressed specifically in the nasal and temporal regions of the developing chick retina, respectively. We previously demonstrated that *FoxG1* controls the expression of topographic molecules including *FoxD1*, and determines the regional specificity of the nasal retina. *FoxD1* is known to prescribe temporal specificity, however, molecular mechanisms and downstream targets have not been elucidated. We addressed the genetic mechanisms for establishing temporal specificity in the developing retina using an *in ovo* electroporation technique (Figure 1A). Fibroblast growth factor (Fgf) and Wnt first play pivotal roles in inducing the region-specific expression of *FoxG1* and *FoxD1* in the optic vesicle. Misexpression of *FoxD1* represses the expression of *FoxG1*, *GH6*, *SOHo1*, and *ephrin-A5*, and induces that of *EphA3* in the retina. *GH6* and *SOHo1* repress the expression of *FoxD1*. In contrast to the inhibitory effect of FoxG1 on bone morphogenic protein (BMP) signaling, *FoxD1* does not alter the expression of *BMP4* or *BMP2*. Studies with chimeric mutants of FoxD1 showed that FoxD1 acts as a transcription repressor in controlling its downstream targets in the retina. Taken together with previous findings, our data suggest that *FoxG1* and *FoxD1* are located at the top of the gene cascade for regional specification along the nasotemporal (anteroposterior) axis in the retina, and *FoxD1* determines temporal specificity (Figure 1B).

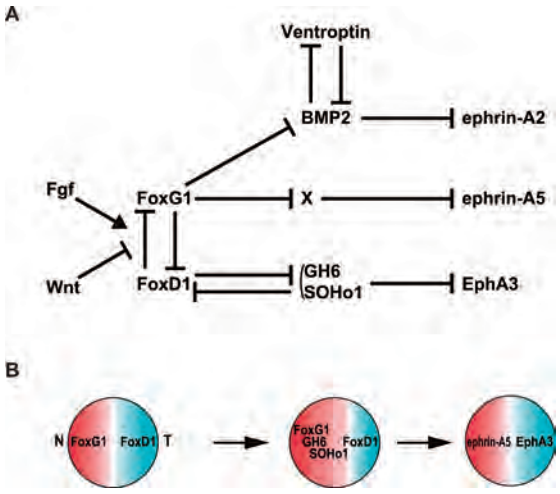


Figure 1. Expressional regulation of asymmetrically distributed molecules along the A–P axis by *FoxG1* and *FoxD1*. (A) The molecular mechanisms by which *FoxG1* and *FoxD1* control the expression of topographic molecules. Fgf8 derived from the anterior neural ridge is required for both *FoxG1* and *FoxD1* expression in the optic vesicle, but an excess of Fgf enhances *FoxG1* expression and represses *FoxD1* expression. Wnt signaling from the mesencephalon inhibits the expression of *FoxG1* and *FoxD1*. *FoxG1* and *FoxD1* counteract each other and *FoxG1* represses the transcription of negative regulators, X. When FoxG1 is absent and FoxD1 is present, X downregulates *ephrin-A5* expression and *FoxD1* represses the expression of *GH6* and *SOHo1*. When FoxG1 is present and FoxD1 is absent, X is downregulated and the expression of *ephrin-A5*, *GH6*, and *SOHo1* is induced. *GH6* and *SOHo1* inhibit the expression of *EphA3*. The inhibitory action of FoxG1 on BMP2 signaling is attributable to the change in the expression of *Ventroptin* and *BMP2* to the oblique-gradient pattern. The counteraction between *Ventroptin* and *BMP2* determines the expression of *ephrin-A2* in an oblique-gradient fashion. Arrows and T-bars indicate positive and negative effects, respectively. These effects are not necessarily induced by direct action. (B) Developmental expression of topographic molecules along the A–P axis. *FoxG1* and *FoxD1* are expressed in a counter-gradient manner in the optic vesicle. After the optic cup forms, the expression domains of *FoxG1* and *FoxD1* are separated by reducing their positive regions to a narrow region of the nasal or temporal retina, respectively. Subsequently, *GH6* and *SOHo1* are expressed specifically in the nasal retina from HH stages 12–14. The expression domains of *GH6* and *SOHo1* fill the gap between the expression areas of *FoxG1* and *FoxD1*. *EphA3* and *ephrin-A5* are homogeneously expressed in the retina

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

at early developmental stages and become restricted to the temporal and nasal retinae, respectively, under the control of these transcription factors at later stages. Development proceeds from left to right. Nasal (N) is left, and temporal (T) is right in the retina.

II. Mechanisms for retinotectal projection

Special attention is now devoted to the molecular mechanisms for axon branching and arborization and their selective elimination. Among the region-specific molecules in the developing retina, we have already identified several molecules which induce abnormal branching and arborization when their expression was experimentally altered *in vivo*.

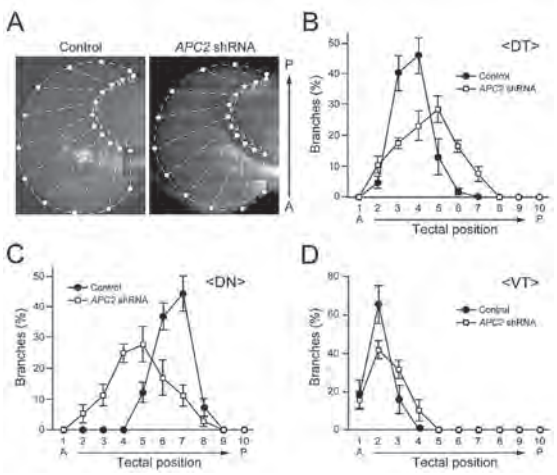


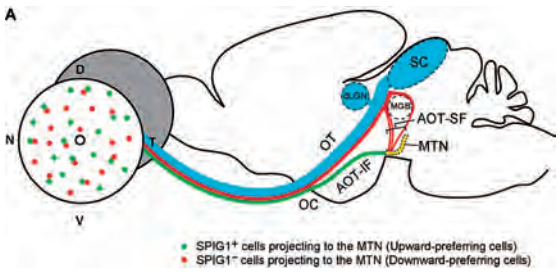
Figure 2. Distribution of interstitial branches of retinal axons on the tectum at E12.5. (A) The tectum was subdivided into 10 areas along the anteroposterior axis, and numbers of branches in each area were counted. (B–D) Quantification of branch distributions of dorsotemporal (DT), dorsonasal (DN), and ventrotemporal (VT) retinal axons, respectively. The values are shown as the mean ±SEM. In APC2 knockdown embryos, interstitial branches from the axon shaft were more diffusely distributed along the anteroposterior axis. A, Anterior; P, posterior.

Growth cones at the tip of growing axons are key cellular structures that detect guidance cues and mediate axonal growth. An increasing number of studies have suggested that the dynamic regulation of microtubules in the growth cone plays an essential role in growth cone steering. The dynamic properties of microtubules are considered to be regulated by variegated cellular factors but, in particular, through microtubule-interacting proteins. We examined the functional role of adenomatous polyposis coli-like molecule 2 (APC2) in the development of axonal projections by using the chick retinotectal topographic projection system. APC2 is preferentially expressed in the nervous system from early developmental stages through to adulthood. Immunohistochemical analysis revealed that APC2 is distributed along microtubules in growth cones as well as axon shafts of retinal axons. Overexpression of APC2 in cultured cells induced the stabilization of microtubules, whereas the knockdown of *APC2* in chick retinas with specific short hairpin RNA reduced the stability of microtubules in retinal axons. *APC2* knockdown retinal axons showed abnormal growth attributable to a reduced response to ephrin-A2 *in vitro*. Furthermore, they showed drastic alterations in retinotectal projections without making clear target zones in the tectum *in vivo* (Figure 2). These results suggest that APC2 plays a critical role in the development of the nervous system through the regulation of microtubule stability.

III. Development of retinal ganglion cell subtypes

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

The direction of image motion is coded by direction-selective (DS) ganglion cells in the retina. Particularly, the ON DS ganglion cells project their axons specifically to terminal nuclei of the accessory optic system (AOS) responsible for optokinetic reflex (OKR). We recently generated a knock-in mouse in which SPIG1 (SPARC-related protein containing immunoglobulin domains 1)-expressing cells are visualized with GFP, and found that retinal ganglion cells projecting to the medial terminal nucleus (MTN), the principal nucleus of the AOS, are comprised of SPIG1$^+$ and SPIG1$^-$ ganglion cells distributed in distinct mosaic patterns in the retina (Figure 3A, left). We examined light responses of these two subtypes of MTN-projecting cells by targeted electrophysiological recordings. SPIG1$^+$ and SPIG1$^-$ ganglion cells respond preferentially to upward motion and downward motion, respectively, in the visual field (Figure 3B). The direction selectivity of SPIG1$^+$ ganglion cells develops normally in dark-reared mice. The MTN neurons are activated by optokinetic stimuli of only vertical motion as shown by Fos expression analysis. Combination of genetic labeling and conventional retrograde labeling revealed that axons of SPIG1$^+$ and SPIG1$^-$ ganglion cells project to the MTN via different pathways (Figure 3A). The axon terminals of the two subtypes are organized into discrete clusters in the MTN. These results suggest that information about upward and downward image motion transmitted by distinct ON DS cells is separately processed in the MTN, if not independently. Our findings provide insights into the neural mechanisms of OKR; how information about the direction of image motion is deciphered by the AOS.



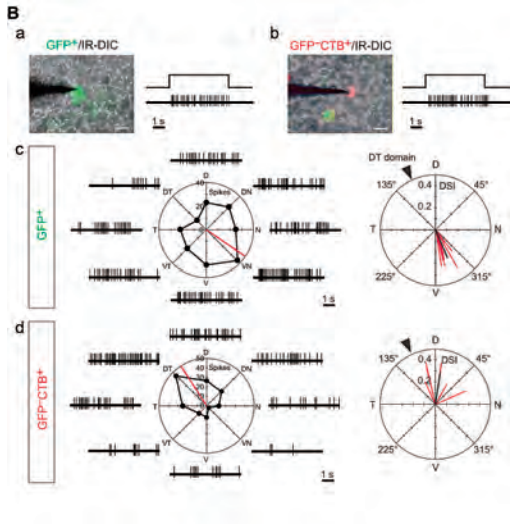


Figure 3. (A) Schematic representation of axonal connectivity between the retina and the contralateral MTN of the AOS. Information of upward and downward visual motion is conveyed to the MTN by distinct neuronal pathways. This represents our findings together with those in the previous studies on the retinal projection to the MTN in mice and rats. Upward-preferring subtype of ON DS cells (SPIG1$^+$; green) predominantly projects to the MTNd via AOT-IF, whereas downward-preferring subtype of ON DS cells (SPIG1$^-$; red) projects to the MTNv via AOT-SF. The fibers of the AOT-SF split from the OT and the brachium of the SC, then they course ventrally over the surface of the cerebral peduncle (CP) and finally terminate in the MTNv. On the other hand, the fibers of the AOT-IF leave the OT just after passing through the optic chiasm (OC), then course caudally, and terminate in the MTNd. MGB: medial geniculate body (B) SPIG1 marks the upward-preferring subtype of ON DS cells. (a, b) *Left*, the tip of a tungsten electrode was attached onto a GFP$^+$ MTN-projecting (MTN-P) cell (a) or GFP$^-$ MTN-P cell (b) to record spike discharges from the cell. Cells were identified by using IR-DIC optics. *Right*, responses of a GFP$^+$ MTN-P cell (a) and GFP$^-$ MTN-P cell (b) to a light stimulus for 5 s. Both showed sustained responses during the light stimulation. (c, d) *Left panels*, representative spike trains and their polar plots in response to eight different directions of the drifting square-wave gratings for 6 s. Spikes were recorded from a GFP$^+$ MTN-P cell (c), GFP$^-$ MTN-P cell (d), respectively. The red line indicates the preferred direction of the cells. *Right panels*, preferred direction and direction selectivity index (DSI) of individual cells are represented by the angle and length of the red line, respectively. The black line indicates the average of the preferred direction and DSI. The DSI indicates the degree of asymmetry in the polar plot of responses to the drifting gratings. The arrowhead indicates the direction of the dorsotemporal domain. Scale bars: a, b, 20 μm.

IV. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein tyrosine phosphatase receptor type Z (Ptprz, also known as PTPζ/RPTPβ) is preferentially expressed in the CNS as a major chondroitin sulfate proteoglycan. Three splicing variants, two receptor isoforms (Ptprz-A/B) and one secretory isoform (Ptprz-S) are known. Receptor-type Ptprz interacts with the PSD95 family through its intracellular carboxyl-terminal PDZ-binding motif in the postsynaptic density. *Ptprz*-deficient adult mice display impairments in spatial and contextual learning. We are conducting experiments to know molecular and cellular mechanisms for the altered phenotypes in *Ptprz*-deficient mice.

V. Mechanisms of Na-level sensing in the brain for body-fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids. For Na homeostasis of the body, control of Na and water intake and excretion are of prime importance. Although it was suggested that the circumventricular organs (CVOs) are involved in body-fluid homeostasis, the system for sensing Na levels within the brain, which is responsible for the control of Na- and water-intake behavior, has long been an enigma.

Na_x is an atypical sodium channel that is assumed to be a descendant of the voltage-gated sodium channel family. Our studies on *Na_x*-knockout mice revealed that Na_x channels are localized to the CVOs and serve as a sodium-level sensor of body fluids. *Na_x*-knockout mice do not stop ingesting salt when dehydrated, while wild-type mice avoid salt. Very recently, we found that *Na_x*-deficient mice show normal vasopressin response to dehydration.

Publication List

〔Original papers〕

- Shintani, T., Ihara, M., Tani, S., Sakuraba, J., Sakuta, H., and Noda, M. (2009). APC2 plays an essential role in axonal projections through the regulation of microtubule stability. J. Neurosci. *29*, 11628-11640.
- Takahashi, H., Sakuta, H., Shintani, T., and Noda, M. (2009). Functional mode of FoxD1/CBF2 for the establishment of temporal retinal specificity in the developing chick retina. Dev. Biol. *331*, 300-310.
- Toychiev, A.H., Sabirov, R.Z., Takahashi, N., Ando-Akatsuka, Y., Liu, H., Shintani, T., Noda, M., and Okada, Y. (2009). Activation of maxi-anion channel by protein tyrosine dephosphorylation. Am. J. Physiol. - Cell Physiol. *297*, C990-C1000.
- Yonehara, K., Ishikane, H., Sakuta, H., Shintani, T., Nakamura-Yonehara, K., Kamiji, N.L., Usui, S., and Noda, M. (2009). Identification of retinal ganglion cells and their projections involved in central transmission of information about upward and downward image motion. PLoS ONE *4*, e4320.

〔Review articles〕

- Noda, M., Takahashi, H., and Sakuta, H. (2009). Neural patterning: Eye fields. *In* Encyclopedia of Neuroscience, Squire, L.R., ed. (Elsevier), pp. 199-204.
- Sakuta, H., Suzuki, R., and Noda, M. (2009). Retrovirus vector-mediated gene transfer into the chick optic vesicle by *in ovo* electroporation. *In* Electroporation and Sonoporation in Developmental Biology, Nakamura, H., ed. (Springer), pp. 105-116.

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

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

The neocortex emerged in mammals and evolved most remarkably in the primate. To understand the underlying mechanisms, we studied gene expression patterns within different areas of the neocortex. Over the last ten years we have reported the findings that are schematically illustrated in Figure 1.

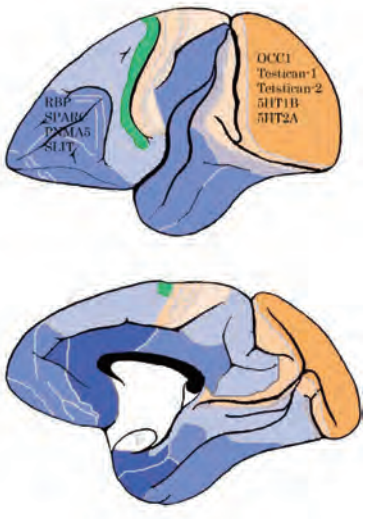

Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

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

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

II. Paraneoplastic Antigen-Like 5 Gene (PNMA5) is preferentially expressed in the association areas in a primate specific manner

As mentioned above, this year we reported enriched expression of the paraneoplastic antigen-like 5 gene (PNMA5) in prefrontal and sensory association areas but not in primary sensory areas, with the lowest expression level in the primary visual cortex. In situ hybridization in the primary sensory areas revealed PNMA5 mRNA expression is restricted to layer II: That is, along the ventral visual pathway, the expression gradually increased in the excitatory neurons from the primary to higher visual areas. This differential expression pattern was very similar to that of retinol-binding protein (RBP) mRNA, another association-area-enriched gene which we reported previously (Figure 2). Additional expression analysis for comparison of other genes in the PNMA gene family, PNMA1, PNMA2, PNMA3, and MOAP1 (PNMA4), showed that they were widely expressed across areas and layers, but without the differentiated pattern of PNMA5. In mouse brains, PNMA1 was only faintly expressed and PNMA5 was not detected. Sequence analysis showed divergence of PNMA5 sequences among mammals. These findings suggest that PNMA5 acquired a certain specialized role in the association areas of the neocortex during primate evolution (Takaji et al., Cereb Cortex. 2009, 19: 2865 - 2879).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*). Members with # belong to SRPBS (Strategic Research Program for Brain Science), NIPS.

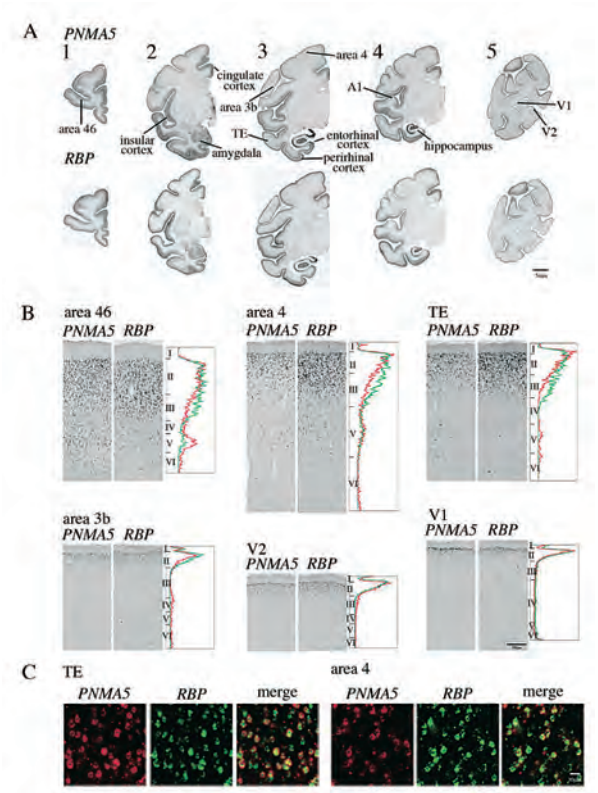

Figure 2. Comparison of expression patterns of PNMA5 and RBP mRNAs in macaque brains. (A) Coronal sections at positions A, C, D, E, F in Figure 2 are shown. (B) Higher magnification views of several areas. The expression patterns of PNMA5 and RBP mRNAs are shown, together with the laminar profiles of ISH signals quantified by measuring the optical density (red, PNMA5 mRNA; green, RBP mRNA). These profiles show the averages of the normalized values of two or three different macaques. The cortical layers were identified in reference to the Nissl staining of the nearby section. (C) Double ISH of PNMA5 and RBP mRNAs were performed to examine the coexpression of these mRNAs. Note that PNMA5 mRNA (left, red) is expressed in a large population of RBP-mRNA-positive cells (middle, green) in layer II. (cited from Takaji et al. Cereb Cortex. 19: 2865 - 2879)

III. Expression of immediate-early genes represents anatomical compartments within ocular dominance columns after brief monocular deprivation. Neocortical areas revealed by layer specific gene expression in rats

The primary visual cortex (V1) of primates is subdivided into compartments reflecting different neural circuits. Visual inputs from the two eyes activate alternating bands of cortex, thereby forming the well-studied ocular dominance columns (ODCs). In addition, the enzymatic reactivity of cytochrome oxidase (CO) reveals "blob" structures within the supragranular layers of ODCs. This year, we presented evidence for compartments within ODCs which have not previously been clearly defined (Takahata et al., 2009, 106:12151-12155). These compartments are revealed by the activity-dependent mRNA expression of immediate-early genes (IEGs), zif268 and c-fos, after brief periods of monocular deprivation (MD). After a 1-3 hr period of MD produced by an injection of tetrodotoxin, IEGs were expressed in a patchy pattern that included infragranular layers, as well as supragranular layers, where they corresponded to the CO blobs. In addition, the expressions of IEGs in layer 4C were especially high in narrow zones along boundaries of ODCs, termed here, the "border strips" of the ODCs. After longer periods of MD (> 5 hr), the border strips were no longer apparent. When the MD was produced by a brief period of monocular eyelid suture, changes in IEG expressions were not evident in layer 4, however, the patchy pattern of the expression that was obvious in infragranular and supragranular layers remained. These changes of IEG expression after MD indicate that cortical circuits involving the CO blobs of the supragranular layers include aligned groups of neurons in the infragranular layers, and that the border strip neurons of layer 4C are highly active for 3 hr after MD (Figure 3).

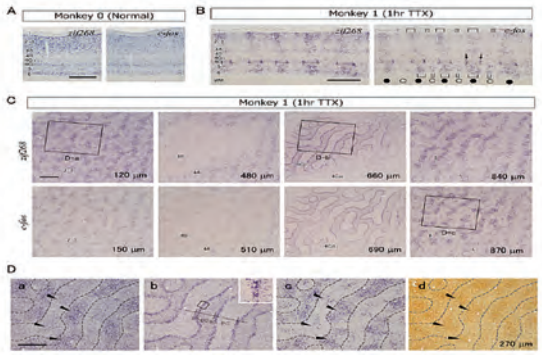

Figure 3. A, B: Coronal sections of ISH for IEGs, zif268 and c-fos, in V1 of Monkey 0 (normal; A) and Monkey 1 (1 hr TTX; B). IEG mRNA expression decreased in the deprived columns (open circles in B) after MD. Note that the widths of strong IEG mRNA expression (black brackets) are thinner than the actual widths of nondeprived columns (filled circles), and that the IEG mRNA expression remains in the center of deprived columns (open brackets). In addition, strong IEG mRNA signals were observed around the border of ODCs in layer 4Cβ (black arrows). Scale bar = 500 μm (A), and 1 mm (B), C: Tangential sections of ISH for IEGs in V1 of Monkey 1. The depth of the section from the pial surface is indicated on each panel. Sections are arranged by increasing depth. Scale bar = 1 mm, D: High magnification views of rectangle windows in C (a-c) and their adjacent section of superficial layer (270 μm away from the pia) stained for CO reactivity (d). Broken lines in a, c and d indicate the boundary of ODCs with reference to the patterns in b. Black arrowheads on the photomicrographs indicate the locations of CO blobs. Compartments of border strips (BSs), dark columns (DCs) and pale columns (PCs) are indicated in b. Insert in b represents a magnified view of the cortex within the rectangle area, at a single cell resolution. Scale bar = 500 μm (cited from Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155)

From these findings, we propose a model of the functional architecture of macaque V1 (Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155; Fig 4A). The functional unit that is seen in CO preparation in the supragranular layers extends to layer 6, with the exception of layer 4C. In addition, border strips reside in the vicinity of the boundaries of ODCs in layer 4C. As for the ODCs, the normally cryptic border strips are revealed by IEG mRNA expression after MD with TTX injection. Border strips of the left and right

eye column adjoin each other with the boundary of ODCs between them. Previously, Horton described CO blobs that included layers 5/6 in macaques (Horton & Hubel, 1981), and the existence of dim CO blobs in infragranular layers has been suggested for squirrel monkeys and prosimian galagos (Carroll & Wong-Riley, 1984; Condo & Casagrande, 1990)). Horton and his colleagues have also suggested the existence of border strips along ODCs in layer 4C in terms of a gap between anterograde tracer signals from the open eye and CO enzymatic activity after long-term MD by eye enucleation (Horton & Hocking, 1998), and pale stripes in layer 4C in CO staining in the strabismus monkeys (Horton, Hocking and Adams, 1999). Although there has been no subsequent evidence for this architecture, our data strongly supports their model except for their suggested absence of a blob structure in layer 4A, as our data indicates that the blob structure also includes layer 4A.

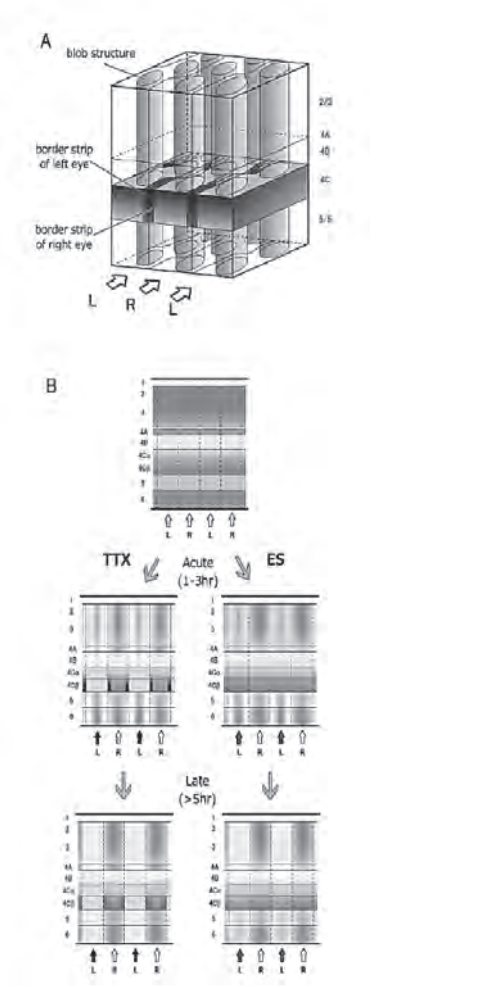

Figure 4. A: Our schematic model of V1 architecture. Blob structure extends from layer 2 to layer 6, with the exception of layer 4C. In addition, there is a border strip structure in the vicinity of the boundaries of ODCs in layer 4C. Although we did not detect strong IEG mRNA signals in layers 4B and 4Cα, previous reports can also be referred for the structure (12, 27). B: Our schematic view of changes of IEG expression, showing the distribution of somas of active neurons after MD (this case, deprivation of the left eye) by TTX or ES. In MD by TTX injection, IEG mRNA expression decreases in interblob regions in both columns, especially in deprived columns, and increases in border strips in nondeprived columns. After a period of time, IEG mRNA expression eventually levels out within each column. This sequential pattern is mostly similar in MD by ES, except that IEG mRNA expression hardly changes in layer 4C. L/projection from left eye, R/projection from right eye (cited from Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155).

Publication List

〔Original papers〕

- Moroni, R.F., Inverardi, F., Regondi, M.C., Watakabe, A., Yamamori, T., Spreafico, R., and Frassoni, C. (2009). Expression of layer-specific markers in the adult neocortex of BCNU-Treated rat, a model of cortical dysplasia. Neuroscienc. *159*, 682-691.
- Takahata, T., Higo, N., Kaas, J.H., and Yamamori, T. (2009). Expression of immediate-early genes reveals functional compartments within ocular dominance columns after brief monocular inactivation. Proc. Natl. Acad. Sci. USA *106*, 12151-12155.
- Takaji, M., Komatsu, Y., Watakabe, A., Hashikawa, T., and Yamamori, T. (2009). Paraneoplastic Antigen-Like 5 Gene (PNMA5) Is Preferentially Expressed in the Association Areas in a Primate Specific Manner. Cereb. Cortex. *19*, 2865 - 2879
- Watakabe, A. (2009). Comparative molecular neuroanatomy of mammalian neocortex: What can gene expression tell us about areas and layers? Dev. Growth Differ. *51*, 343-354.

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 to research of the visual system, though we had previously been researching the salt-sensing system (~March, 2008).

I. Psychobiological study of medaka fish

One of our major subjects is the psychobiological study of medaka (*Oryzias latipes*). Medaka, as well as zebrafish, have many advantages for behavioral work. First, genetic examination of medaka is progressing at a rapid pace, like that of the mouse, opening up new approaches to the understanding of genetic control of behavior. Second, although the central nervous system of medaka is relatively simple, its basic structure is the same as that in mammals; it is composed of the spinal cord, brainstem, cerebellum, and cerebrum. Thirdly, because they are fish, they provide invaluable comparative material for work on mammals. Examination of such a relatively simple yet vertebrate system should thus aid in the determination of the basic mechanisms of how genes affect behavior.

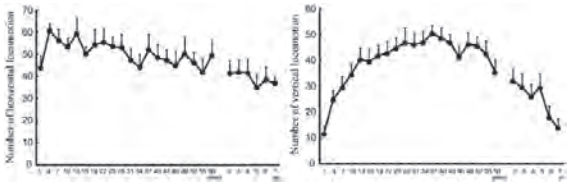

Figure 1. Temporal changes in horizontal (left) and vertical (right) locomotion of medaka fish in an open field in 3-min bins.

This year, we examined the habituation of medaka to a novel environment by measuring long-term temporal changes in locomotion by using open-field apparatus. The open-field test is commonly used in behavioral studies of rodents. However, it has not been used in most behavioral studies of fish. We therefore examined the open-field behavior of medaka as measured by temporal changes in two conventional indices, locomotion and position. Considering the characteristic motility of fish, we focused on vertical as well as horizontal locomotion. In 7-hour as well as 24-hour exposure of fish to the open field, the pattern of temporal changes in vertical locomotion differed from that of horizontal locomotion (Figure 1). During repeated open-field testing over two consecutive days, locomotion on the second day was less than that on the first day. This habituation-like behavior ceased with the addition or deletion of a visual cue on the second day. We also found that males and females differed in temporal patterns of habituation to the open field. These findings clearly show that medaka change their behavior as they become more familiar with a novel location, as observed in ethological studies of other animal species. The open-field test of medaka will thus provide findings of use in elucidating abnormal phenotypes of mutant medaka.

II. Psychophysical study of human vision

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

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

One recent focus of this debate is the flash-lag effect, in which a moving object is perceived to lead a flashed object when both objects are aligned in actual physical space. The discoverer of this phenomenon, Dr. Nijhawan, has proposed that the human visual system uses motion signals to extrapolate the position of a moving object. The differential latencies hypothesis proposes that the flash-lag effect occurs simply because the visual system responds with a shorter latency to moving objects than to flash stimuli. Besides these two major models, various modified models have been proposed. The problem, however, has not yet been solved, and the debate continues. How does our brain decide the position of the moving object? We are now making an attempt to correctly interpret the flash-lag effect by developing a novel motion illusion (Kabob illusion, Figure 2).

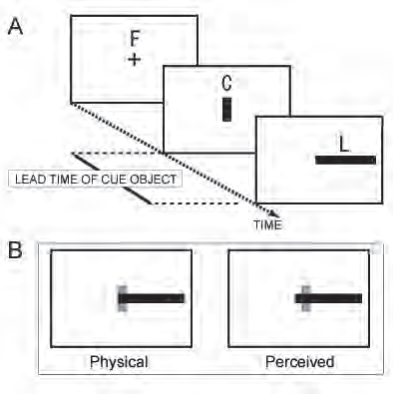

Figure 2. Kabob illusion. A: Sight line of observer is fixed on a central cross (F). A fixed time after the cross disappears, a cue (C) appears in the center. After an appropriate time interval (lead time of cue object), a line (L) is presented. Observer perceives illusory motion from cue side toward the opposite side of the line (line-motion effect). B: When the line-motion effect occurs, a leading cue (gray) is perceived to move along the line (solid).

LABORATORY OF NEUROCHEMISTRY

Associate Professor
SASAOKA, Toshikuni

NIBB Research Fellow: *SATO, Asako*

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

I. Role of dopaminergic transmission in locomotion and eating behavior

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

In mammals, two subgroups of dopamine receptor have been identified, referred to as D1-like receptors (D1R, D5R) and D2-like receptors (D2R, D3R and D4R) on the basis of their gene structure and their pharmacological and transductional properties. D1R and D2R are the most abundantly and widely expressed in the brain and often play a synergistic role. In collaboration with Dr. Motoya Katsuki, Executive Director, National Institute of Natural Sciences, We generated *D1R/D2R* double knockout (DKO) mice and observed that *D1R/D2R* DKO mice exhibited severe impairment in locomotion, no initiation of eating, and died by 4 weeks of age.

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

II. Locomotor activity controlled by D1R expression

To elucidate the effects of changing *D1R* expression led by Dox administration, we applied Dox for two weeks and monitored daily locomotor activity and food/water intake (Figure 1). In *D1R/D2R* DKO background, mice exhibited decreases in locomotion and food/water intake as well as a decrease in the amount of transgene expression after Dox administration. After withdrawal of Dox administration, the mice exhibited transient hyperactivity and then recovered locomotor activity and food/water intake (Figure 2). We further analyzed locomotor activities of transgenic mice in other backgrounds and found that hyperactivity after withdrawal of Dox administration was unique to mice in *D1R/D2R* KO and *D1R* KO background, which have no endogenous *D1R* expression.

We also examined the protein expression level of D1R in the striatum of transgenic mice in *D1R/D2R* DKO background. The striatum is considered to be a major region responsible for control of locomotor activity and containing abundant *D1R* expression. During the process of recovery of locomotor activity after Dox withdrawal, the transgene D1R expression gradually increased while locomotor activity fluctuated strikingly. These results indicate that the increment of locomotor activity is not simply in proportion to the amount of D1R expression. Instead, increase of D1R expression from an abnormally low level is critical. To understand mechanisms of locomotor control through D1R signaling, we are analyzing the relationship between the *D1R* expression and altered behavior. In addition, we are investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

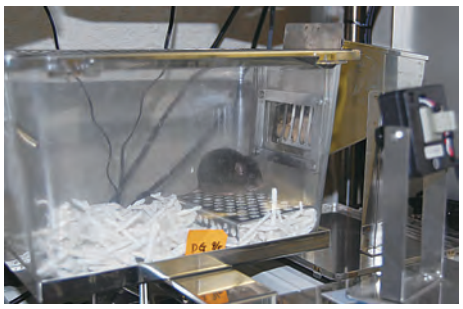

Figure 1. Experimental equipment for measurement of locomotor activity and food/water intake

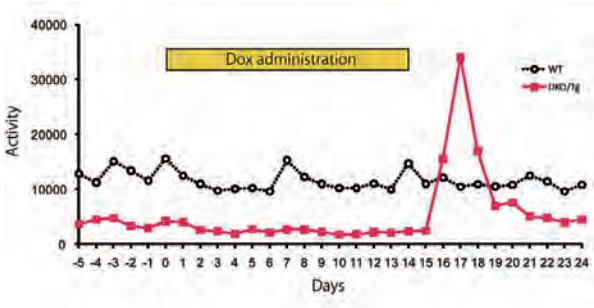

Figure 2. Locomotor activity of *D1R* transgenic mice that have *D1R/D2R* DKO background. After withdrawal of Dox administration, transient hyperactivity was observed.

Publication List

〔Original paper〕

● Tao, H., Suzuki, M., Kiyonari, H., Abe, T., Sasaoka, T., and Ueno, N. (2009). Mouse prickle1, the homolog of a PCP gene, is essential for epiblast apical-basal polarity. Proc. Natl. Acad. Sci. USA, *93*, 2110-2115.

DIVISION of GENOME DYNAMICS

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	WATANABE, Taka-aki
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	OKAMOTO, Haruko
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	YONEZAWA, Harumi
	MATSUZAKI, Yoko
Secretary:	*MIKAMI, Yuriko*

The genomes of higher organisms contain significant amounts of repetitive sequences which, in general, are unstable. At present, neither the physiological function(s) of these repeated sequences, nor the mechanism producing repeated sequences and controlling instability are fully understood. To clarify these aspects, we are pursuing several themes using *Saccharomyces cerevisiae*, and Chinese Hamster Ovary (CHO) cells. In 2009 we have made progress in understanding a new role of condensin in maintaining the long repeated structure of rDNA, in which it is an essential step that condensin is recruited to the *RFB* (replication fork barrier) site in rDNA repeat units by three protein factors (Tof2p, Csm1p and Lrs4p) and Fob1 protein. In addition, we also constructed a new system of gene amplification via DRCR (double rolling circle replication) in yeast by using Cre-*lox* site-specific recombination. Furthermore we constructed an analogous system for the CHO chromosome and found that it produced gene amplification products also seen in tissue cultured cells under natural conditions.

Previously we constructed *Eschericia coli* cells with a linear genome and found they behave like *E. coli* with a circular genome (Cui *et al*., EMBO Rep. *8*, 181-187, 2007). We investigated how the genome behaves when the circular genome is linearized by TelN protein.

I. Mechanism of condensin recruitment onto the RFB site located within rDNA repeats in budding yeast

The primary functions of chromosome condensation during cell division are to facilitate the individualization of sister chromatids, which remain entangled after DNA replication, and to shorten the length of chromatid arms so that they avoid truncation during chromosome segregation to opposite poles of the cell. The compaction ratio of mitotic chromosomes relative to double stranded DNA ranges from ~160-fold in budding yeast to ~10000-20000-fold in mammalian chromosomes. Despite its being a fundamental process for cell growth, we have a surprisingly poor understanding of the higher levels of chromosome organization. Many textbooks feature the radial loop model of condensed mitotic chromosomes in which the chromatin fibers are bunched around a chromosome scaffold; this model is largely based on electron microscopy of mitotic chromosomes under high-salt or polyanion conditions. One clue for understanding the structure of condensed chromosomes was the identification of components of the chromosomal scaffold, namely, Top2 and condensin. Although, both of these are known to be required for building up condensed mitotic chromosomes, their relationship to specific DNA elements is still poorly understood. Condensin is a multi-subunit protein complex that plays a central role in mitotic chromosome condensation and segregation. In vertebrates, condensin is distributed in the axial part over the whole length of condensed chromosomes, but this has only been shown at the resolution of light microscopy. The sites where condensin acts on the chromatin and the molecular mechanisms of condensin recruitment onto thereto had largely remained elusive. In previous studies, we found that condensin localized to the *RFB* site in a Fob1 (fork block protein) dependent manner during S-phage. To date, this Fob1p-dependent condensin localization is the only example of condensin association with a specific DNA site in a specific protein factor-dependent manner. With the goal of understanding chromosome condensation at the molecular level, we have studied mechanisms of condensin localization at the *RFB* site. This year we discovered that condensin can bind to a short DNA fragment containing *RFB* sequences, even if the sequence was inserted at an ectopic chromosomal site. This indicates that the *RFB* site itself acts as a site recruiting onto chromatin. Analysis of the relationship between condensin recruitment to the *RFB* site and Fob1p-dependent replication fork blockage at the *RFB* site demonstrated that those two events were completely independent phenomena. Instead, we identified three additional protein factors, Tof2p, Csm1p, and Lrs4p, necessary for both *FOB1*-dependent condensin recruitment to the *RFB* site and for ensuring the faithful segregation of long rDNA repeats. We also found ordered binding of Fob1p, Tof2p, Csm1p/Lrs4p, and condensin complex at the *RFB* site, as shown in Figure 1. Finally, *in vivo* interaction between Csm1p, Lrs4p and multiple subunits of condensin were detected. These results suggest that condensin is recruited to the *RFB* site by the sequential interaction of Fob1p, Tof2p, Csm1p, Lrs4p, and finally condensin, to ensure the proper segregation of long rDNA tandem array.

II. Condensin contributes to repressing transcriptions by RNA polymerase II in the rDNA cluster

As described above, we found that the replication fork barrier (*RFB*) site, which is located in the intergenic spacer region (IGS) within the ribosomal RNA gene (rDNA) cluster, worked as a cis element for condensin recruitment to chromatin. Interestingly, recruitment of condensin to the RFB site occurs in the DNA synthesis-phase, long before mitotic-phase, suggesting that condensin plays some role during interphase. We are investigating the mechanism of condensin recruitment to chromatin and its role in maintaining the integrity and the higher order structure of the rDNA repeated region. This year, we discovered that condensin contributed to silencing of RNA polymerase II

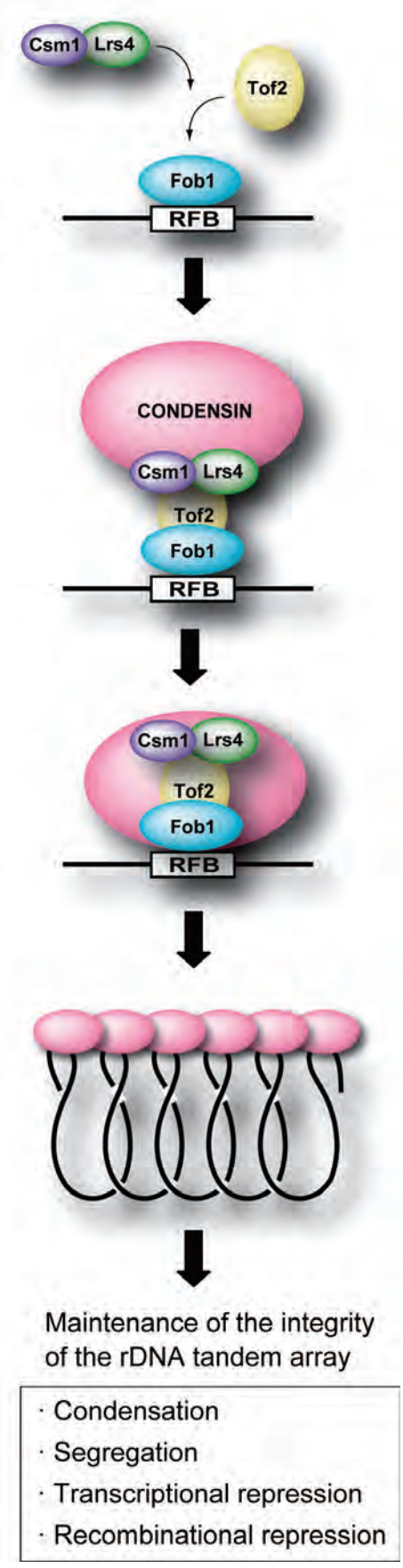

Figure 1. Model for condensin recruitment to the *RFB* site, contributing to maintenance of the integrity of long rDNA repeats.

transcription from a reporter gene inserted close to the RFB site. Condensin mutation leads to a decrease in the Sir2 association profile in rDNA. As well as the changes in the Sir2 association profile, endogenous transcriptions by RNA polymerase II within the IGS region increased in condensin mutants. Based on these observations, we hypothesize that condensin has an effect on the higher-order chromatin structures of the IGS region, resulting in the promotion of Sir2 accessibility to the region, thus contributing to transcriptional silencing.

III. Construction of a new gene amplification system via DRCR (double rolling circle replication) using Cre-lox site specific recombination in budding yeast and CHO (Chinese hamster ovary) cell

In addition to rDNA gene amplification in eukaryotes, there is another type of gene amplification, which is involved in various biological phenomena, such as cancer development and drug-resistance. However, the mechanism is largely unknown because of the complexity of the amplification process. Previously, we developed a gene amplification system in *S. cerevisiae* that is based on double rolling-circle replication (DRCR), utilizing break-induced replication (BIR). This system produced two types of amplification products. Type-1 products contain 13 to ~100 copies of the amplification marker, *lue2d* (up to 730 kb increase) with novel arrangement present as randomly oriented sequences flanked by inverted *leu2d* copies. Type-2 products are acentric multi-copy mini-chromosomes, each carrying two *lue2d* copies. Structures of type-1 and -2 products resemble those of homogeneously staining regions (HSRs) and double minutes (DMs) of higher eukaryotes, respectively. Interestingly, products analogous to theses were generated at low frequency without deliberate DNA cleavage.

If DRCR were an actual gene amplification mechanism in yeast, a quite different initiation reaction, which can induce DRCR, should produce amplification products resembling HSRs and DMs. Thus, we tried to construct a new DRCR amplification system that is induced by another process; Cre-*lox* site-specific recombination. Cre-*lox* site-specific recombination occurs between un-replicated and replicated regions during replication, the fork replicates these already-replicated regions again, and the Cre recombination system makes this process more efficient. Furthermore, a combination of the process efficiently induces gene amplification through DRCR. In fact this system produced two kinds of products; the structures of these products resemble those of HSRs and DMs of higher eukaryotes, respectively. Furthermore, the same genetic elements produce HSR- and DM-like products when placed in cultured mammalian (CHO) cells. Thus, we concluded that DRCR is indeed an amplification mechanism in lower and higher eukaryotes and can be naturally initiated if some structural requirement is satisfied.

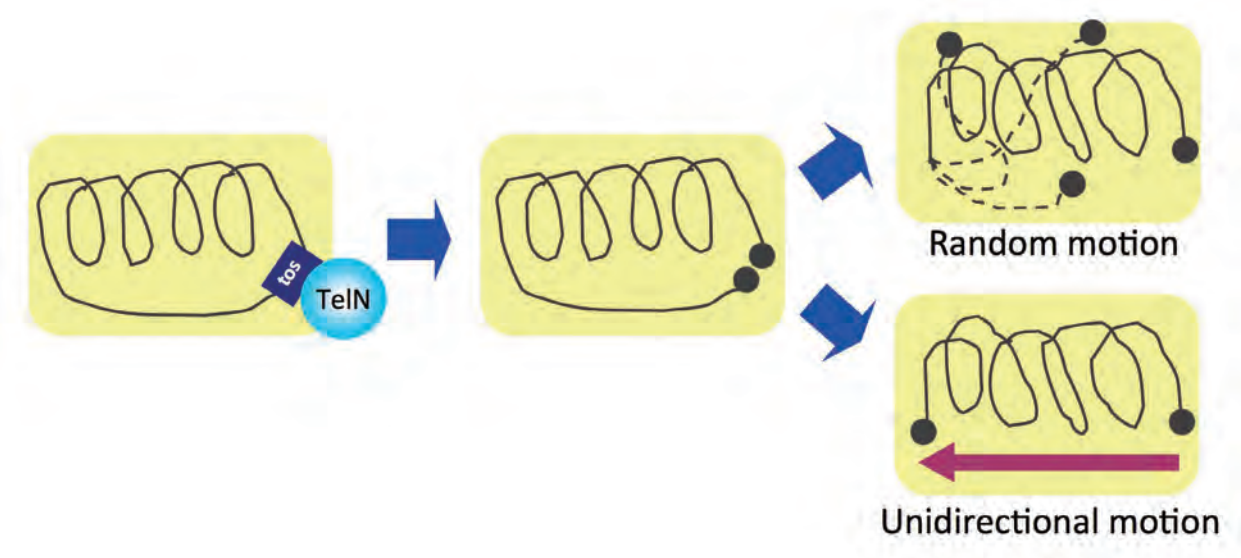

Figure 2. How do two termini of *E. coli* genome behave when the circular genome is linerized?

IV. *E. coli* with linear genome: how do two termini of the genome behave when the circular genome is linearized?

Previously, we constructed *E. coli* with a linear genome (Cui *et al*., EMBO Rep. *8*, 181-187, 2007). Interestingly, the *E. coli* strain with a linear genome did not show any distinct characteristics in comparison with circular genome *E. coli*. Here, we investigate how each end of the linear genome behaves when the circular genome is linerized with TelN enzyme (linearization enzyme coded in N15 lysogenic phage genome). We expected two possibilities; one was that each end would move randomly, at least for a while, the other was that each end would separate to each pole position of the cell (Figure 2).

In order to know which possibility was correct, the following *E. coli* strain was constructed; *tos* sequence, a target sequence for TelN enzyme, was inserted into the site within the replication terminus region (the site close to *dif*), an opposite region of replication origin (*oriC*) of the circular genome. Then, at both sides of *tos*, two kinds of operator (*tetO* and *lacO*) repeated sequences were inserted. Finally, CFP-*tet* and YFP-*lac* repressor genes were inserted downstream of arabinose promoter, and *telN* gene was positioned downstream of a rhamnose promoter on the *E. coli* genome.

The strain was grown in M9-succinate medium logalismic phase and then arabinose was added to the medium to induce CFP-*tet* and YFP-*lac* repressors, which bind to the *tetO* and *lacO* sites, respectively, close to each other before linearization. Then, by addition of rhamnose to the culture, TelN enzyme was induced, which cut the *tos* site and converted the circular genome to a linear one. During these experiments, we examined the behavior of two fluorescent spots under a microscope. The observations show that the two terminuses, after linearization, do not move randomly, but one stays in place and the other tends to move to the other pole site of the cell. Average rate of the latter type of movement is approximately 0.216μ/min. Because the DNA is neither dramatically stretched nor shrunk, these results strongly suggest participation of proteins.

Publication List

〔Original paper〕

- Johzuka, K., and Horiuchi, T. (2009). The *cis*-element and factors required for condensin recruitment to chromosome. Mol. Cell *34*, 26-35.

DIVISION OF MOLECULAR GENETICS †

Professor
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	TSUGANE, Kazuo
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	EUN, Chang-Ho
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Visiting Scientist:	*YAMAUCHI, Takaki*
Technical Assistants:	*ONO, Akemi*
	TSUGANE-HAYASHI, Mika
	SHIMATANI, Zenpei
	SAITOH, Hiromi
	ASAO, Hisayo
	MATSUMOTO, Miwako
	SHIMAMOTO, Miki
	MATSUDA, Chisato
	HASEGAWA, Yoshinobu
Secretary:	*SANJO, Kazuko*

The main interest of this division was characterizing various aspects of genetic and epigenetic gene regulations including the flower pigmentation of morning glories. In addition, we were undertaking reverse genetic approaches in order to elucidate the nature of dynamic genomes in rice, a model plant for cereals.

Because the activities of this group were terminated at the end of March, please consult the last year's annual report for most of our activities with the exception of the following knock-in targeting of a rice gene by homologous recombination.

I. Knock-in targeting of endogenous natural genes by homologous recombination in rice

Although the analysis of the expression of a reporter gene fused with an appropriate promoter segment in transgenic plants, often termed promoter-reporter gene fusion analysis, is widely used to characterize the cloned promoter sequences, inter-individual variation of the reporter gene expression among transgenic plants, which is attributed mainly to the insertion sites and/or copy number of the transgene and epigenetic gene silencing, hampers proper spatiotemporal evaluation of the promoter activity. To circumvent such problems, an approach known as a "promoter trap" is also employed; a transformant having a promoterless reporter gene, which is carried by appropriately modified T-DNA sequences or transposons, integrated into an exon of an endogenous target gene in the proper orientation and resulting in the reporter gene expression by transcriptional fusion, is to be isolated for promoter analysis among the transformants containing the randomly inserted transgenes. Because the promoter trap is based on random insertional mutagenesis, the isolation of an appropriate promoter-trapped mutant relies on a fortuitous integration of the transgene into the target gene of interest, even though the promoter trap is much more likely to accurately reflect the expression of a gene than promoter-reporter gene fusion. Moreover, simultaneous integration of additional transgene copies may cause a potential problem because multiple insertions may complicate the interpretation of spatiotemporal expression patterns. As is the case in mice, the homologous recombination-promoted knock-in-targeting strategy is expected to be a powerful tool to monitor promoter activity accurately in plants. Knock-in targeting appears to offer at least two advantages over the promoter trap; the junction sequence between a reporter gene and an endogenous target promoter can be designed properly, and transgenic plants carrying an identical and desired knock-in allele can be repeatedly obtained. We have succeeded in obtaining 15 independent and fertile transgenic knock-in rice plants, which have only one copy of the promoterless *GUS* reporter gene encoding β-glucuronidase fused with the endogenous promoter of *MET1a*, one of two rice *MET1* genes encoding a maintenance DNA methyltransferase. As Figure 1 shows, the spatiotemporal gene expression of *GUS* was reproducibly observed in a dosage-dependent manner among independently isolated plants.

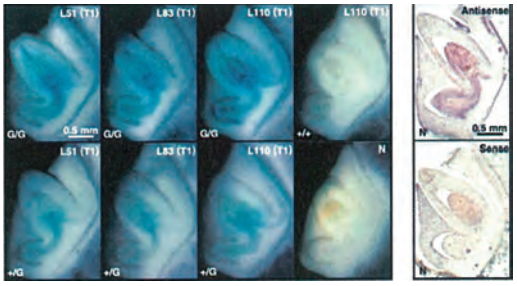

Figure 1. Reproducible and dosage-dependent expression of *GUS* in knock-in targeted plants. (**Left**) Embryos in T1 plants. (**Right**) *In situ* hybridization pattern of *MET1a* in embryos of Nipponbare. The symbols G/G, +/G, and +/+ indicate *GUS/GUS*, *MET1a/GUS*, and *MET1a/MET1a*, respectively.

Publication List

〔Original papers〕

- Hoshino, A., Park, K.I., and Iida, S. (2009). Identification of *r* mutations conferring white flowers in the Japanese morning glory, *Ipomoea nil*. J. Plant Res. *122*, 215-222.
- Ikeda-Kawakatsu, K., Yasuno, N., Oikawa, T., Iida, S., Nagato, Y., Maekawa., M., and Kyozuka, J. (2009). Expression level of *ABERRANT PANICLE ORGANIZATION* determines rice inflorescence form through control of cell proliferation in the meristem. Plant Physiol. *150*, 736-747.
- Shimatani, Z., Takagi, K., Eun, C.H., Maekawa, M., Takahara, H., Hoshino, A., Qian, Q., Terada, R., Johzuka-Hisatomi, Y., Iida, S., and Tsugane, K. (2009). Characterization of autonomous *Dart1* transposons belonging to the *hAT* superfamily in rice. Mol. Gen. Genomics *281*, 329-344.
- Yamauchi, T., Johzuka-Hisatomi, Y., Fukada-Tanaka, S., Terada, R., Nakamura, I., and Iida, S. (2009). Homologous recombination-mediated knock-in targeting of the *MET1a* gene for maintenance DNA methyltransferase reproducibly reveals dosage-dependent spatiotemporal gene expression in rice. Plant J. *60*, 386-396.

†: This laboratory was closed on 31 March, 2009.

DIVISION OF EVOLUTIONARY BIOLOGY

Professor
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	TSUKAMOTO, Naho
	SAKURAI-OZATO, Nami
	WASHIO, Midori
	YAMADA, Hiroko
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Secretary:	*KOJIMA, Yoko*

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

I. Evolution from unicellular to multicellular organisms

The first evolutionary step from unicellular to multicellular organisms is the formation of two different cells from a single cell via asymmetric cell division. The first cell division of a protoplast isolated from the protonemata of the moss *Physcomitrella patens* is asymmetric regarding its shape and nature, and gives rise to an apical pluripotent stem cell and a differentiated protonema cell. A systematic overexpression screening for genes involved in asymmetric cell division of protoplasts in *P. patens* was performed for 4,000 full-length cDNA clones. We identified 58 cDNAs whose overexpression caused defects in asymmetric cell divisions and their functional analyses are in progress. This work was performed as a collaboration with Dr. Tomomichi Fujita (Hokkaido University).

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

The cells of land plants and their sister group, charophycean green algae, divide by the insertion of cell plates at cytokinesis. This is in contrast to other green algae, in which the invagination of the plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards the cell periphery, and finally fuses to the parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. Centrifugal expansion of the phragmoplast is a driving force for that of the cell plate, although elucidating the molecular mechanism for the centrifugal expansion of the phragmoplast was a challenge. Based on live imaging of α-tubulin at a light microscopic level and immunolocalization of γ-tubulin at an electron microscopic level, we proposed a hypothesis that cytosolic γ-tubulin complexes are recruited onto existing phragmoplast microtubules and nucleate new microtubules as branches, and that the branched microtubules drive phragmoplast expansion. Seeing the life history of microtubules in the phragmoplast had been very difficult by live imaging of α-tubulin, but we successfully tracked the trajectories of growing microtubule ends in the phragmoplast using two-photon microscopy of a microtubule plus-end marker EB1. Microtubules appeared in many sites in the phragmoplast and elongated obliquely towards the cell plate. We also found that inhibition of γ-tubulin function by antibody injection inhibited formation of new microtubules and phragmoplast expansion. These results support our hypothesis. Takashi Murata was this study's main researcher.

Microtubules form arrays with parallel and antiparallel bundles and function in various cellular processes including cell division. The interdigitation of antiparallel microtubules in phragmoplasts, plant-unique microtubules arrays, is formed and stabilized by microtubules-associated proteins (MAPs) including kinesins. However, mechanisms for disassembly of the interdigitation of antiparallel microtubules are still unclear. Here we show a type II ubiquitin-like domain protein, PUBL1 and PUBL2 (for *Physcomitrella patens ubiquitin-like domain protein 1 and 2*), are novel factors regulating disassembly of the antiparallel bundles and depolymerization of the microtubules in the phragmoplasts. PUBL1 and PUBL2 proteins were predominantly localized to the interdigitation of the antiparallel microtubules. In the double-deletion lines of both genes, the collapse of the interdigitating microtubules in the phragmoplasts was retarded, indicating that PUBL1 and PUBL2 are indispensable for proper loss of the interdigitation. A kinesin, KINID1a, for generation of the interdigitation aberrantly persisted in the phragmoplast equator, suggesting that the crosslink between the plus ends of the antiparallel microtubules is properly lost in the double-deletion lines. Furthermore, double-deletion lines exhibited formation of incomplete cell plate and multinucleated cells, suggesting that PUBL1 and PUBL2, the proper disassembly of the interdigitation, or both are required for proper lateral expansion of the cell plate. Yuji Hiwatashi mainly performed this study.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

III. Evolution of molecular mechanisms in plant development

3-1 Stem cell initiation and maintenance

The initiation and maintenance of several types of stem cells to produce different types of differentiated cells is precisely regulated during the development of multicellular organisms. Molecular mechanisms for stem cell characterization, however, have remained largely unknown. We showed that *AINTEGUMENTA/PLETHORA/BABY BOOM (APB)* orthologs *PpAPB*s (*PpAPB1*, *2*, *3*, and *4*) are involved in stem cell characterization in the moss *Physcomitrella patens*. Gametophore stem cells were induced by exogenous cytokinin in the wild type, while the quadruple disruptants did not form any gametophore stem cells with exogenous cytokinin application. These results suggest that the *PpAPB*s play a critical role in the characterization of a gametophore stem cell. Meanwhile, the expression of *PpAPB*s is regulated by auxin, not cytokinin. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

3-2 Nuclear genome project of the moss *Physcomitrella patens*

A comparison of developmental genes among major land plant taxa would facilitate our understanding of their evolution, although it has not been possible because of the lack of genome sequences in basal land plants. We established an international consortium for a genome project of the moss *Physcomitrella patens* and the lycopod *Selaginella moellendorffii*. After publication of the draft genome of *Physcomitrella patens*, to further elaborate the contig assembling and the gene annotation, we performed (1) EST analyses of several libraries of cDNAs isolated from different developmental stages; (2) construction of full-length cDNA libraries and sequencing in their full length; (3) construction of BAC libraries and their end-sequencing; (4) 5'-end serial analysis of gene expression (5' SAGE); and (5) a collection of 3' UTR and small RNA sequences as collaborative works with groups associated with Dr. Tomoaki Nishiyama (Kanazawa Univ.), Prof. Asao Fujiyama (National Institute of Informatics), Prof. Sumio Sugano (Univ. Tokyo), and Prof. Yuji Kohara (National Institute of Genetics).

We developed a system to efficiently construct phylogenetic trees with whole genome shotgun sequence data in public databases before their assembly. We collected homologs of approximately 700 *Arabidopsis thaliana* genes involved in development, and their phylogenetic analyses are in progress.

3-3 Functional characterization of polycomb genes in the moss *Physcomitrella patens*

Land plants have distinct developmental programs in haploid (gametophyte) and diploid (sporophyte) generations. Although usually the two programs strictly alternate at fertilization and meiosis, one program can be induced during the other program. In a process called apogamy, cells of the gametophyte other than the egg cell initiate sporophyte development. Here, we report for the moss *Physcomitrella patens* that apogamy resulted from deletion of the gene orthologous to the *Arabidopsis thaliana CURLY LEAF* (*PpCLF*), which encodes a component of polycomb repressive complex 2 (PRC2). In the deletion lines, a gametophytic vegetative cell frequently gave rise to a sporophyte-like body. This body grew indeterminately from an apical cell with the character of a sporophytic pluripotent stem cell but did not form a sporangium. Furthermore, with continued culture, the sporophyte-like body branched. Sporophyte branching is almost unknown among extant bryophytes. When PpCLF was expressed in the deletion lines once the sporophyte-like bodies had formed, pluripotent stem cell activity was arrested and a sporangium-like organ formed. Supported by the observed pattern of PpCLF expression, these results demonstrate that, in the gametophyte, PpCLF represses initiation of a sporophytic pluripotent stem cell and, in the sporophyte, represses that stem cell activity and induces reproductive organ development. In land plants, branching, along with indeterminate apical growth and delayed initiation of spore-bearing reproductive organs were conspicuous innovations for the evolution of a dominant sporophyte plant body. Our study provides insights into the role of PRC2 gene regulation for sustaining evolutionary innovation in land plants. Yosuke Okano and Takaaki Ishikawa were this study's primary researchers.

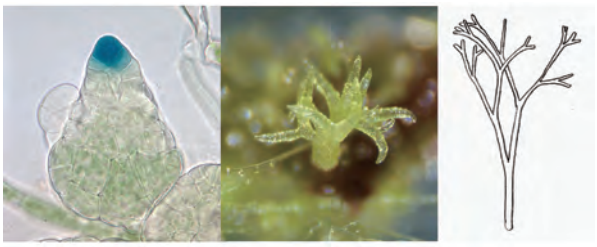

Figure 1. A *PpCURLY LEAF* deletion mutant formed sporophyte-like bodies with expression of a sporophyte stem cell specific MKN4 gene (left). A sporophyte-like body formed branches (center), which is similar to extinct protracheophytes (right).

IV. Molecular mechanisms of female and male interactions

In sexual reproduction, proper communication and cooperation between male and female organs and tissue are essential for male and female gametes to unite. In flowering plants, female sporophytic tissues and gametophyte direct a male pollen tube towards an egg apparatus, which consists of an egg cell and two synergid cells. The cell-cell communication between the pollen tube and the egg apparatus, such as the reception of a signal from the egg apparatus at the pollen tube, makes the tip of pollen tube rupture to release the sperm cell. To detect male factors involved in this communication, we screened mutants of receptor-like kinases expressed in pollen tubes and characterized the *ANXUR1* (*ANX1*) and *ANXUR2* (*ANX2*) genes. Here we report that pollen tubes of *anx1*/*anx2* ruptured before arriving at the egg apparatus, suggesting that *ANX1* and *ANX2* are male factors controlling pollen tube

behavior by directing rupture at the proper timing. Furthermore, *ANX1* and *ANX2* were the most closely related paralogs to a female factor, *FERONIA*/*SIRENE*, controlling pollen tube behavior expressed in synergid cells. Our finding shows that the coordinated behaviors of female and male reproductive apparatuses are regulated by the sister genes, whose duplication might play a role in the evolution of fertilization system in flowering plants. This work was mainly done by Saori Miyazaki.

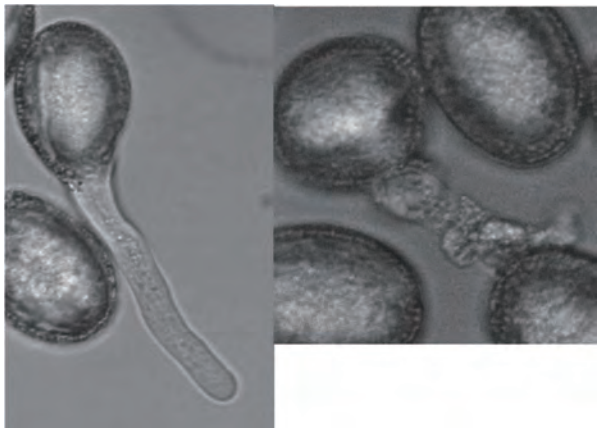

Figure 2. Pollen tubes of wild type (left) and the *anx1;anx2* mutant (right) on culture medium. Ruptured *anx1;anx2* pollen tube released sperm nuclei together with pollen tube cytoplasm before fertilization.

V. Molecular mechanisms of mimicry

Mimicry is an intriguing phenomenon in which an organism closely resembles another, phylogenetically distant species. An excellent example is the flower-mimicry of the orchid mantis *Hymenopus coronatus*, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. To elucidate the evolutionary mechanism of this complex mimicry at the molecular level, we first focused on the mechanism of body coloration in the orchid mantis. HPLC and mass spectrometric analyses suggested that xanthommatin, a red pigment belonging to the ommochrome family, contributes to the pink body coloration of the mantis. We also found that the orchid mantis contains large amounts of leucopterin and isoxanthopterin, both of which are known as white compounds in other insects such as *Pierid* butterflies. These results indicate that the "flower-like" coloration of the orchid mantis is formed by pigments unrelated to those used for the coloration of flowers.

The orchid mantis drastically changes its appearance during post-hatching development. The first-instar nymph of the mantis is colored red and black and is believed to mimic other unpalatable insects like ants. A flower-like appearance emerges after molting into the 2nd-instar nymph. We aim to compare the gene expression profiles between the 1st- and 2nd-instar nymphs using a high-throughput DNA sequencer. This work was mainly done by Hiroaki Mano.

VI. Molecular mechanisms of host shifting

Although plant-feeding insects as a whole utilize various plant species, the majority of plant-feeding insect species are associated with one or a few plant species. Such mono- and oligophagous insect species are highly specialized to their respective host plant species via larval physiological adaptation (assimilability) and host preference of adult females. Thus, the process of host shifting to a novel plant species involves the evolution of multiple traits. The molecular mechanisms underlying such multi-trait evolution are largely unknown. To address the molecular mechanism of host shifting, we use two host races in a tiny moth, *Acrocercops transecta*, as a model system. Host races in plant-feeding insects are subpopulations that are specialized to different species of host plants, so we can conduct QTL analyses of the host-adaptation traits by crossing the two host races. The segregation patterns of larval assimilability and ovipositing female preference in F2 and backcross generations indicated that the two traits were governed by a few major loci, but were under different genetic control. To test whether these loci are physically linked with each other or not, mapping analyses are in progress. This study was conducted mainly by Issei Ohshima.

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DIVISION OF SYMBIOTIC SYSTEMS

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More than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. AM fungi absorb minerals, mainly phosphates, from the soil and provide them to the plants. The origin of AM symbiosis is thought to have been in the early Devonian period. On the other hand, the root nodule symbiosis that occurs between legumes and rhizobial bacteria, unlike AM symbiosis, involves host-specific recognition and postembryonic development of a nitrogen-fixing organ. Root nodule symbiosis is thought to have evolved about 60 million years ago. Despite marked differences between the fungal and bacterial symbioses, common genes required for both interactions have been identified using model legumes. Our laboratory, which was launched in April 2009, focuses on the early stages of the interaction between these microorganisms and *Lotus japonicus* in order to reveal the molecular mechanism and the origin of these symbiotic systems.

I. Long-distance control of nodulation

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

1-1 Exploration for root-derived long-distance signal(s) involved in autoregulation of nodulation

Host legumes control root nodule numbers by sensing external and internal cues. A major external cue is soil nitrate, whereas a feedback regulatory system in which earlier formed nodules suppress further nodulation through shoot-root communication is an important internal cue. The latter, AON, is believed to consist of two long-distance signals: a root-derived signal that is generated in inoculated roots and transmitted to the shoot; and a shoot-derived signal that systemically inhibits nodulation. In *L. japonicus*, the leucine-rich repeat receptor-like kinase, HAR1, mediates AON and nitrate inhibition of nodulation, and is hypothesized to recognize the root-derived signal in the shoot.

Among 39 *L. japonicus CLE* genes, we identified *LjCLE-RS1* and *LjCLE-RS2* as strong candidates for the root-derived signal. These genes are not expressed in shoots but strongly up-regulated in the rhizobial-inoculated roots. A time course analysis for gene expression revealed that the induction of *LjCLE-RS1* and *-RS2* started to up-regulate 3 hours after rhizobial inoculation and the timing for the induction of those genes was earlier than that for the initiation of the regulatory response of nodulation that is detectable 3 days after rhizobial inoculation in *L. japonicus*. By using the hairy root transformation method, in the wild-type background, we showed that overexpressing *LjCLE-RS1* and *-RS2* inhibits nodulation and this effect was also observed in non-transformed roots, whereas overexpressing *LjCLE3* did not repress nodulation (Figure 1). By contrast, in the *har1* mutant background, overexpressing *LjCLE-RS1* and *-RS2* did not inhibit nodulation. Thus, overexpressing *LjCLE-RS1* and *-RS2* inhibits nodulation systemically and this effect depends on HAR1 receptor-like kinase.

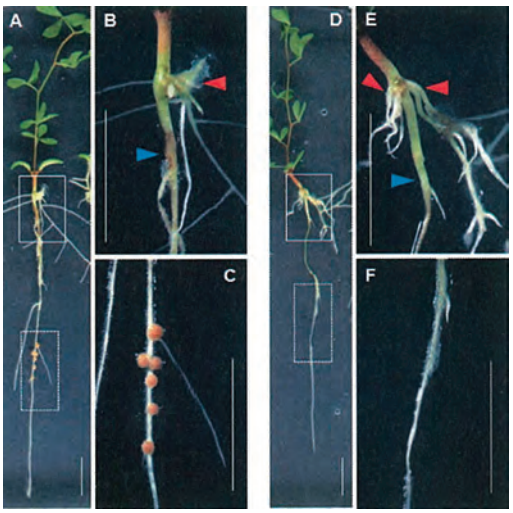

Figure 1. Wild type plants possessing hairy roots overexpressing *LjCLE3* (A to C) and overexpressing *LjCLE-RS1* (D to F). Blue arrowheads indicate normal untransformed root system. Red arrowheads indicate the initiation site of the hairy root system. Bars = 1 cm.

Nitrogen depletion in the soil is a prerequisite for nodule development and function, and high concentrations of nitrogen as nitrate or ammonia abolishes nodulation. Mutations in *HAR1* exhibit nitrate-tolerant symbiotic phenotype. Through expression analysis using 39 *LjCLE* genes, we found that *LjCLE-RS2* was strongly and specifically up-regulated in the root in response to nitrate. Exposure of seedlings to different nitrate concentrations showed that the level of *LjCLE-RS2* transcript accumulation increased by adding 1 mM KNO_3 and reached a maximum at

30 mM KNO_3, a concentration that interferes with *L. japonicus* nodulation. Based on the finding, we proposed a model in which nitrate-induced LjCLE-RS2 inhibits nodulation via HAR1 receptor-like kinase.

1-2 TML, a root regulator associated with the long-distance control of nodulation

too much love, tml, is a novel hypernodulating mutant isolated by C^{6+} beam mutagenesis of the seeds of *L. japonicus* Miyakojima MG-20. To locate the potential site of action of *TML*, we conducted reciprocal grafting experiments with the wild type and *tml* mutants. The seedlings were used for wedge grafting surgery and the successful grafts were transferred to vermiculite and inoculated with *Mesorhizobium loti*. Grafting a *tml* shoot onto a wild-type root led to nodulation in the wild-type; in contrast, grafting a wild-type shoot onto a *tml* root resulted in an increased number of nodules/nodule primordia, which was indistinguishable from that of *tml* self-grafts. This root-determined hypernodulation of *tml* indicates that unlike *HAR1* and *KLV*, *TML* functions in the roots rather than in the shoots.

The role of *TML* in the roots but not in the shoots prompted us to ask whether a root factor *TML* and a shoot factor *HAR1* genetically interact with each other despite the different sites of action. For this purpose, we carried out reciprocal grafting using *tml* and *har1-7* mutants. We confirmed that the hypernodulation of *har1-7* is regulated by the shoots, consistent with the previous studies using different *har1* alleles. This shoot-regulated *har1-7* hypernodulation was not obviously enhanced by grafting a *har1-7* shoot onto a *tml* root, suggesting that *TML* and *HAR1* might constitute the same long-distance signaling. On the other hand, grafting a *tml* shoot onto a *har1-7* root complemented the hypernodulation of each other, further supporting the specific roles of *TML* and *HAR1* in the roots and the shoots, respectively.

Based on these findings, at least two potential mechanisms by which a root factor TML exerts its inhibitory effect on nodulation can be speculated: TML might perceive or mediate an unknown shoot-derived signal produced by HAR1, or TML might generate or relay a root-derived signal. To examine which hypothesis is more valid, we designed inverted-Y grafting, where a sliced root is grafted into a short slit made in a stock plant. The inverted-Y grafting experiments suggested that the effect is likely to be local, supporting the former hypothesis that TML might function downstream of HAR1.

II. Arbuscular mycorrhiza symbiosis

In the arbuscular mycorrhiza (AM) symbiosis, plant roots accommodate Glomeromycota fungi within an intracellular compartment, the arbuscule. At this symbiotic interface, fungal hyphae are surrounded by a plant membrane, which creates an apoplastic compartment, the periarbuscular space (PAS) between fungal and plant cell. Despite the importance of the PAS for symbiotic signal and metabolite exchange, only few of its components have been identified. Part of this reason is that the AM developmental process does not show clear morphological changes like nodule formation. Having no clear check point of the inoculation process makes it difficult to characterize responses to AM impregnation in the host plant.

In order to solve the problem, we took advantage of AM gene marker *SbtM1* and *LjPT4* isolated in *L. japonicus*. Subtilisin-like serine protease *SbtM1* and phosphate transporter *LjPT4* were induced soon after contact of the fungal hyphae to the host epidermal cells and showed the strongest induction in arbuscule containing cells. Suppression of *SbtM1* gene function by RNAi caused low AM colonization in the host root. This result indicates SbtM1 has an essential role in AM development. *LjPT4* was isolated as a homolog of *MtPT4*. Knockdown of *MtPT4* expression was reported to cause premature death of arbuscules, meaning that PT4 is also required for AM development. Considering the AM specific expression patterns and the importance of SbtM1 during AM development, we employed these genes as an indicator of AM inoculation. Promoter GUS fusion showed strong activation of the promoter by infection of AM fungi (Figure 2A-C) but they were not induced in symbiosis mutants. Fluorescent protein fusion with SbtM1 enabled visualization of the AM inoculation process (Figure 2D, E). Genetic screening of symbiosis mutants which abolished AM specific signaling and regulation system is in progress. The AM specific makers would be a powerful tool to analyze AM phenotypes in novel mutants.

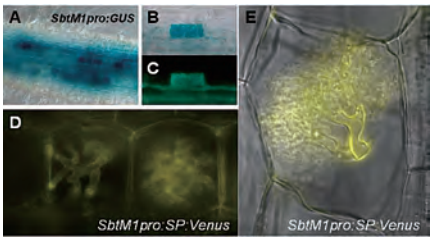

Figure 2. Transformed *L. japonicus* roots containing *SbtM1* promoter:GUS showed staining in response to AM inoculation (A). Stronger GUS staining was observed in arbuscule containing cells compared with the neighboring cells (B and C). AM fungi were stained with WGA Alexa 488 (C). SbtM1 signal peptide:Venus fusion visualized intraradical fungal structures in the host root (D and E).

III. Mathematical models of shoot apical meristem

The shoot apical meristem (SAM) of plants contains stem cells that have the ability to renew themselves and differentiate all aerial tissues such as stems and leaves. the SAM consists of a central zone (CZ) and its surrounding area named the peripheral zone (PZ) that is induced by an unknown signal from the CZ. Maintenance of the SAM essentially involves the interaction between WUS and CLV, in which WUS activates itself and CLV, but CLV inhibits WUS expression. While *clv* mutants show enlarged SAM and stems with fasciation and dichotomous branching, *wus* mutants generate a flattened structure of the SAM because of reduced SAM activity but produce many ectopic shoots to

generate bushy plants after prolonged incubation.

Since it is not clarified how the SAM controls its proliferation and patterning, we constructed and analyzed a mathematical model of the SAM that includes conditions of WUS-CLV dynamics, PZ induction by CZ, and area expansion by cell division. In numerical simulations, the SAM maintains a constant cell number under the wild type condition because of the balance between increase of cells by cell division and departure from the SAM to the outer region (Figure 3). Whereas strong *clv* mutation results in an enlarged and elongated SAM, in weak *clv* mutants the SAM initially elongates and then divides into two independent SAMs. These phenotypes correspond to fasciation and dichotomous branching of stems in *clv* mutants. This model successfully generates the wild type and *clv* mutant phenotypes.

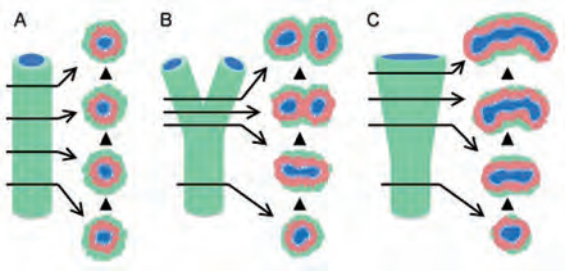

Figure 3. Numerical simulations under conditions of the wild type (A), weak (B) and strong (C) *clv* mutations.

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〔Review article〕

- Magori, S., and Kawaguchi, M. (2009). Long-distance control of nodulation: molecules and models. Molecules Cells *27*, 1-10.

LABORATORY OF MORPHODIVERSITY

Associate Professor
KODAMA, Ryuji

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

I. Wing morphogenesis

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

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

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

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

Trachea and tracheoles are both important in delivering air into the wing and their patterns coincide with that of the boundary of degeneration and differentiation zones at the distal end of the wing. According to previous observations, the pattern formation of wing epithelium is often dependent on tracheal and tracheole patterns. Basic research on the development of tracheal pattern formation is being done by scanning and transmission electron microscopy (Figure 1) to describe the exact pathway and time course of the formation of the elaborate pattern of trachea and tracheoles and to establish the cytological and developmental relationship between the formation of the tracheal pattern and the epithelial cell pattern.

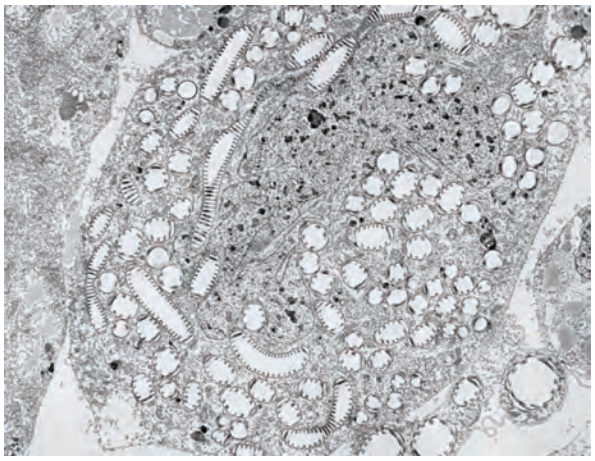

Figure 1. Transmission electron micrograph of the tracheole cell. Many cross-sections of the tracheoles can be observed within the cytoplasm of the tracheole cell. As the cell migrates, the tracheole is laid behind.

II. Other research activities

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

LABORATORY OF BIORESOURCES

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NIBB Research Fellow:	*KIMURA, Tetsuaki*
Research Fellows:	*KANEKO, Hiroyo*
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	TESHIMA, Yuuko
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Secretary:	*SUZUKI, Tokiko*

Teleosts comprise about half of all vertebrate species and have adapted to a variety of environments, including sea-water, fresh water, the bottom of deep seas, small creeks and paddy fields. Analysis of their genome structure is important in order to understand the adaptation and diversification in this interesting group. Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted a comparative genomic analysis focusing mainly on fish chromosomes and gene evolution using medaka and other fishes, and identification of the causal gene of mutants for PGC migration. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

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

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

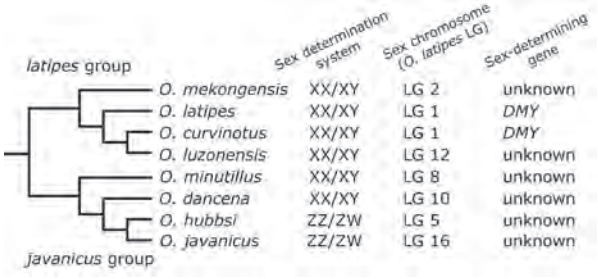

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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

The migratory pathways of PGCs to the gonad vary depending on the vertebrate species, yet the underlying regulatory mechanisms guiding PGCs are believed to be common between species. In the medaka embryo, PGC migration follows two major steps before colonizing in gonadal areas: (1) bilateral lineup in the trunk, and (2) posterior drift. *kazura* (*kaz*) and *yanagi* (*yan*) mutants of medaka isolated in our mutagenesis-screening were defective in the first and second steps, respectively. Positional cloning identified phenotype responsible mutations in the chemokine receptor genes *cxcr4b* and *cxcr7*, respectively. Although belonging to the same chemokine receptor families, involvement of these genes in the regulation of PGC migration was clearly distinct. *cxcr4b* is expressed in the PGCs themselves, suggesting a cell-autonomous function. In contrast, *cxcr7* is not expressed in the PGCs but in the mesoderm-derived tissues connected to the route of PGC migration, the ventral part of the somites in the area always immediately anterior to the PGCs drifting along the bilateral routes toward the gonad, and the pronephric ducts (Sasado et al., 2008). Further analysis of the mutants is underway to reveal the function of the two chemokine-systems in the regulation of PGC migration. *kamigamo* (*kmg*) and *shimogamo* (*smg*) are both recessive lethal mutations showing PGC distribution defects similar to that of *yan* but in different complementation groups (Sasado et al., 2004). Positional cloning of the responsible genes of the mutations is now in progress.

III. The development of insertional mutagenesis system with Tol2 transposon

Mutagenesis screens by N-ethyl-N-nitrosourea have been performed in medaka. However, the cloning of chemically mutated genes is still laborious even after completion of medaka genome sequencing. In order to rapidly identify a causal mutation of phenotype, we undertake insertional mutagenesis in medaka using a transposable element, Tol2.

Tol2 is a transposable element of the hAT transposable element family, residing in the genome of the medaka. The Hd-rR, an inbred strain sequenced in the genome project, has 14 integration sites in its genome. These sites are stably inherited in the Hd-rR.

We demonstrated that Tol2 can be excised in the Hd-rR genome when transposase mRNA, synthesized in vitro, is injected. Multiple different excisions occurred in founder fish. These mutations were highly heritable, however due to the mosaic like nature of the germ cells progeny showed various new mutations. Our results show that Tol2 is useful as a mutagen in medaka.

IV. National BioResource Project Medaka (NBRP Medaka)

4-1 Full length cDNA sequencing project

To establish the full length cDNA resources of medaka we made 11 full length cDNA libraries (developmental stage 22, 35, 40, ovary, testes, brain, male liver, female liver, gill, kidny and spleen) and determined the sequences of both ends of 250,000 clones in collaboration with the National Institute of Genetics' Kohara and Fujiyama labs. Now 499,944 sequences are availabe. After mass alignment of all sequences, we found 21,588 independent sequences at the 3' ends. All of the data was deposited in the DDBJ and is accessible from the National BioResource Project Medaka website (http://www.shigen.nig.ac.jp/medaka/).

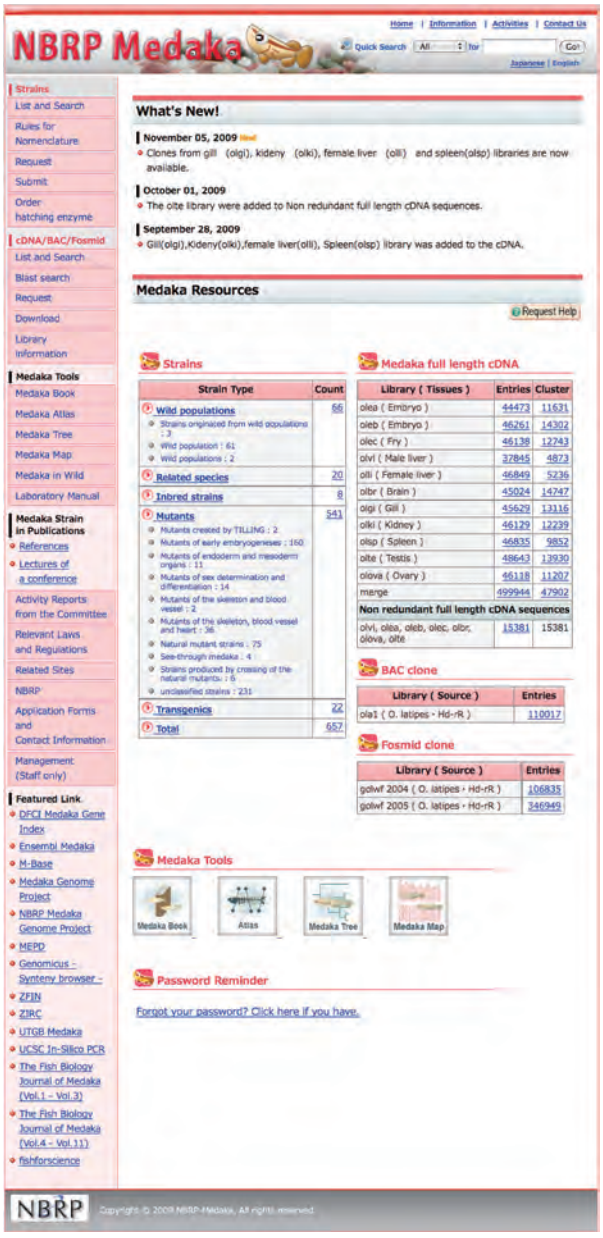

Figure 2. NBRP Medaka website

4-2 Establishment of core facility of NBRP medaka

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

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

- Kato, M., Takehana, Y., Sakaizumi, M., and Hamaguchi, S. A sex-determining region on the Y chromosome controls the sex-reversal ratio in interspecific hybrids between Oryzias curvinotus females and Oryzias latipes males. Heredity. 2009 September 16.

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- Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. (2009). Medaka: Biology, Management and Experimental Protocols. (Wiley-Blackwell, Ames, Iowa).
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- Sasado, T. (2009). Cryopreservation of medaka sperm. In: Medaka: Biology, Management and Experimental Protocols. Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds. (Wiley-Blackwell, Ames, Iowa), pp. 105-110.

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

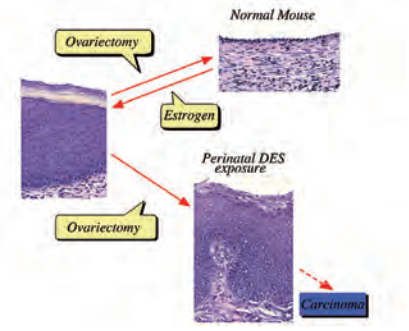

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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

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

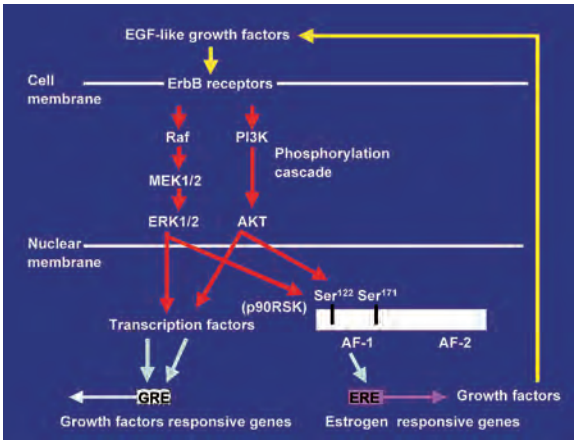

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

II. Microarray analysis of estrogen responsive genes

To understand the mechanisms through which estrogenic chemicals act on mouse reproductive organs, data documenting the temporal and spatial expression patterns of estrogen-responsive genes is essential. A large number of genes affected by estrogen treatment were identified in the tissues of wild-type mice using a microarray approach. For most of the identified genes, expression was induced by 17β-estradiol (E_2) in a dose-dependent manner. Subsequently, several environmental (xeno)estrogens were tested and characteristic gene expression patterns were observed for each compound tested. These patterns were

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

distinct from that obtained following E_2 exposure. We also found that xenoestrogenic chemicals and dioxin have distinct effects on the liver as well. Therefore, possible tissue-specific effects should be considered when elucidating the distinct effects of various EDCs.

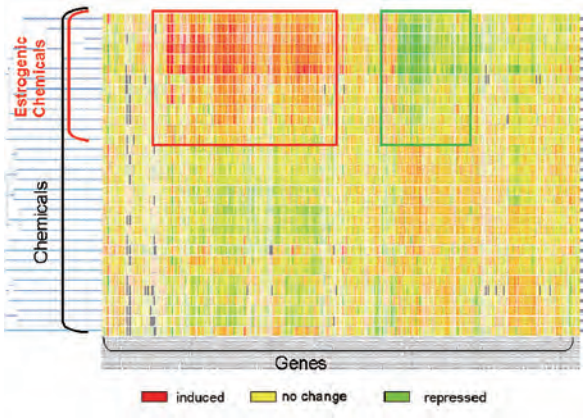

Figure 3. Scatter plot of average expression levels in control and chemical-treated uterus

III. Steroid hormone receptors of birds, reptiles, amphibians and fishes

Steroid and xenobiotic receptors (SXR) have been cloned from various animal species (fish, amphibian, reptiles, birds and mammals) by our group and we have demonstrated species-specific differences in their responses to various environmental and endogenous chemicals (receptor gene zoo). Thus, simple predictions of chemical effects based on data from a few established model species are not sufficient to develop real world risk assessments. ER and ER-like genes have been cloned from various animal species including rockshell, *Amphioxus*, lamprey, lungfish, sturgeon, gar, roach, stickleback, mosquitofish, mangrove, *Rivulus*, various sharks, Japanese giant salamander, Tokyo salamander, newt, axolotl, toad, *Silurana tropicalis*, American alligator, Nile crocodile, freshwater turtle, various snakes and vultures. Functional studies showed that the rockshell ER-like sequence does not bind estrogen but exhibits ligand-independent transactivation, whereas lamprey ER exhibited ligand-dependent transactivation, proving that primitive vertebrates, such as the Agnatha, have a functional ER. Intriguingly, we found that medaka 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.

IV. Male production by juvenile hormones in Daphnids

Daphnia magna has been used extensively to evaluate the organism- and population-based responses of toxicity or reproductive toxicity tests. These tests, however, provide no information about the mode of action

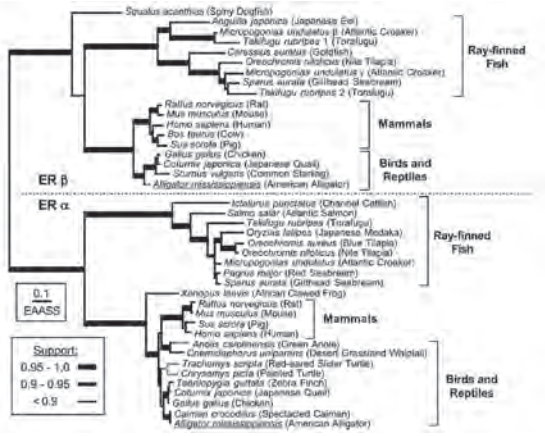

Figure. 4 Evolutionary relationships of estrogen receptor sequences.

of the tested compounds. Therefore, we applied an ecotoxicogenomic assessment of *D. magna*. We established a *Daphnia* EST database and developed an oligonucleotide-based DNA microarray with high reproducibility. Exposure of *D. magna* to several chemicals resulted in characteristic gene expression patterns that are chemical-specific, indicating that the established DNA microarray can be used for the classification of toxic chemicals as well as for the development of a mechanistic 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 observed that juvenile hormone agonists (pesticides), for example, induce the production of male offspring. The molecular basis of environmental sex determination is largely unknown in *D. magna*. Therefore, we isolated sex determination-related genes to understand the molecular mechanisms of this phenomenon in *Daphnia*. DM-domain genes are well known as sex-related genes. We identified four DM-domain genes: DMRT11E, DMRT93B, DMRT99B and DSX. Quantitative gene expression analysis in daphnid gonads revealed that DMRT93B and DSX were expressed only in the testis. We have recently 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.

V. Gene zoo and receptor zoo

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

VI. Molecular target search

We found that the persistent and ubiquitous environmental contaminant, tributyltin chloride (TBT), induces the differentiation of adipocytes *in vitro* and increased adipose mass *in vivo*. TBT is a dual nanomolar affinity ligand for both the retinoid 'X' receptor (RXR) and the peroxisome proliferators activated receptor γ (PPARγ). TBT promotes adipogenesis in the murine 3T3-L1 cell model and perturbs key regulators of adipogenesis and lipogenic pathways *in vivo*. Moreover, *in utero* exposure to TBT leads to strikingly elevated lipid accumulation in the adipose depots, liver, and testis of neonate mice and results in increased epididymal adipose mass in adults. In the amphibian *Xenopus laevis*, ectopic adipocytes form in and around gonadal tissues following organotin, RXR or PPARγ ligand exposure. TBT represents the first example of an environmental EDC that promotes adipogenesis through RXR and PPARγ activation. Developmental or chronic lifetime exposure to orgaotins may therefore act as a chemical stressor for obesity and related disorders. We have recently started to use stem cells from mouse bone marrow to study the molecular mechanisms of cell differentiation to adipocytes by environmental chemicals.

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- Myers, J.P., vom Saal, F.S., Akingbemi, B.T., Arizono, K., Belcher, S., Colborn, T., Chahoud, I., Crain, D.A., Farabollini, F., Guillette, L.J.Jr., Hassold, T., Ho, S.-M., Hunt, P.A., Iguchi, T., Jobling, S., Kanno, J., Laufer, H., Marcus, M., McLachlan, J.A., Nadal, A., Oehlmann, J., Olea, N., Palanza, P., Parmigiani, S., Rubin, B.S., Schoenfelder, G., Sonnenschein, C., Soto, A.M., Talsness, C.E., Taylor, J.A., Vandenberg, L.N., Vandenbergh, J.G., Vogel, S., Watson, C.S., Welshons, W.V., and Zoeller, R.T. (2009). Why public health agencies cannot depend upon good laboratory practices as a criterion for selecting data: The case of bisphenol A. Environ. Health Perspect., *117*, 309–315.

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

I. Mechanisms of leaf development

Focusing on the mechanisms that govern the polarized growth of leaves in *Arabidopsis thaliana,* we have identified four genes for polar-dependent growth of leaf lamina: the *ANGUSTIFOLIA* (*AN*) and *AN3* genes, which regulate the width of leaves, and the *ROTUNDIFOLIA3* (*ROT3*) and *ROT4* genes, which regulate the length of leaves. *AN* and *ROT3* genes control cell shape while *AN3* and *ROT4* genes regulate cell numbers in leaves. In addition to the polar-dependent leaf shape control, we have focused on the mechanisms of organ-wide control of leaf size, which are reflected in the 'compensation' phenomenon (reviewed in Tsukaya 2008). Additionally, the accumulation of knowledge on the basic mechanisms of leaf shape control has enabled us to conduct Evo/Devo studies of the mechanisms behind leaf-shape diversity. Below is an overview of our research activities and achievements during 2009.

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

AN is a member of the *CtBP-BARS* gene family reported from animal genomes; last year, we showed that homologs of AN from *Larix gmelinii,* a gymnosperm, *Marchantia polymorpha* and *Physcomitrella patens,* mosses, all fully complemented all known morphological phenotypes caused by *an-1* mutation in Arabidopsis, suggesting that the AN function is conserved among land plants. Furthermore, our detailed analysis of intracellular localization suggested that AN has a unique role (or roles) in Golgi-related functions. Further analyses of AN functions are ongoing.

On the other hand, we found that constitutive over-expression of ROT4 peptide in Arabidopsis caused abnormal protrusion of the inflorescence stem, suggesting that ROT might be somehow involved in positional value determination.

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

We have recently started to attempt an understanding of the genetic basis of the development of unifacial leaves that are known only from monocot clades. Our analyses indicated that the unifacial character might be due to overall changes in all polarities around leaves (*i.e.* adaxial-abaxial, distal-proximal, and central-lateral polarities). Moreover, the genetic controls of leaf polarities were revealed to differ, at least in part, between eudicots and rice, a monocot model species. Understanding the differences in the genetic mechanisms for the establishment of unifacial and normal bifacial leaves will provide good clues as to how leaf-shape is diversified.

For such purposes, comparative molecular-genetic and anatomical analyses between unifacial and bifacial leaf development have been undertaken using members of the genus *Juncus* (Yamaguchi and Tsukaya, J. Plant Res. 123, 35-41, 2010). Interestingly, molecular characterization of unifacial leaves of *Juncus* revealed that they have only abaxial identity in the leaf blades, lack leaf margins, and possess flattened leaf lamina. Taken together, our data strongly suggests the presence of unknown mechanisms for flat leaf organogenesis that were not previously suspected from studies of model plants. We also established mutational and transgenic approaches to analyze unifacial leaf formation; several interesting mutants of *Juncus* that exhibit abnormalities in leaf polarity have already been isolated.

1-3 Size control of leaves and mechanisms of compensation

We have recently noticed that leaf organogenesis depends on the "leaf meristem" that is seen just in the border region between leaf blade and leaf petiole. All cells required for leaf formation seem to be supplied from this leaf meristem. We also identified that *SPT* controls the size of the leaf meristem (Ichihashi et al., Plant Cell Physiol. 51, 252-261, 2010). How are cell proliferation and cell enlargement coordinated in leaf morphogenesis? In a determinate organ - a leaf - the number of leaf cells is not necessarily reflected in leaf shape or, in particular, in leaf size. Genetic analyses of leaf development in Arabidopsis showed that a compensatory system (or systems) acts in leaf morphogenesis in a way that an increase in cell volume might be triggered by a decrease in cell number (reviewed in Tsukaya 2006; 2008). Thus, leaf size is, at least to some extent, regulated at the organ level by the compensatory system or systems. To understand the details of such totally unknown regulatory mechanisms, we have conducted a large scale screening of leaf-size and/or leaf-shape mutants.

As a result, we have succeeded in isolating *oli* mutants

which have a specific defect in leaf-cell numbers. Using these mutants we revealed that the compensation is triggered only when decrease of cell numbers in leaf primordia exceeds some threshold level; and several *oli* mutations are loss-of-function mutations of ribosome biogenesis genes (Fujikura et al. 2009).

In addition, we have revealed that: (1) *fugu5* phenotype is cancelled by supplying sucrose to the growth medium; (2) large-leaved *gra* mutations are caused by translocation-dependent duplication of a segment of a chromosome (Horiguchi et al. 2009). This enlargement of leaves due to the duplication of the chromosome segment can be explained only partially by the increase of the gene dosage of known gene functions in the chromosome region, suggesting presence of some unknown genes for leaf size control; (3) "opposite-type" compensation syndrome in *msc* mutants was attributed to accelerated heteroblasty (Usami et al., 2009: Fig. 1). Detailed analyses of this phenomenon strongly suggested that traits of heteroblasty are regulated by at least two different pathways governed by small RNAs; (4) a new tool for studies of mechanisms of compensation, inducible, chimeric expression systems of *KRP2* or *AN3*, was established: and (5) several candidate genes responsible for the compensation were selected from microarray analysis of *fugu2* and *an3*.

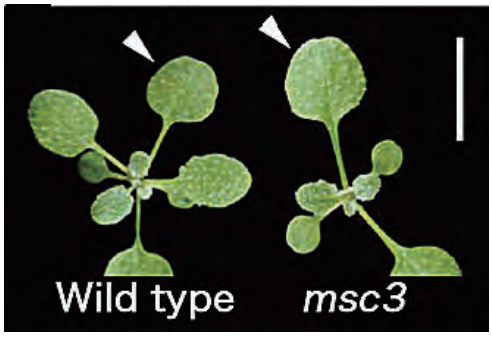


Figure 1. The *msc3* mutant has an altered heteroblasty pattern and develops larger leaf blades that contain a larger number and smaller size of cells, due to disturbance of miR156 function. Modified from Usami et al. (2009).

In addition, in the course of studies of *AN3* function, we found that *an3* mutation phenotype is drastically changed when combined with ribosome-biogenesis mutations and/or #2047 mutation. These facts suggest that AN3 is involved in various key aspects of organogenesis in Arabidopsis. We also found that plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation (Nelissen et al., PNAS *107*, 1678-1683, 2010). Further analyses on the mechanisms of compensation are in progress.

1-4 Size control of leaves and ploidy level

Why does a high-ploidy level cause increased cell/leaf size? In other words, why are tetraploid leaf cells twice as large in volume as diploid leaf cells? The reasons are not yet perfectly understood (Tsukaya 2009). We found that mutational defects in the endoreduplication were responsible for a curious enhancement of the effects of tetraploidization in terms of cell-size increase, suggesting that some unknown mechanisms (*e.g.* feedback systems) are hidden behind the relationship between the ploidy level and cell/organ size. We also found the ratio of cell size between diploid and tetraploid varied among the mutants examined, suggesting that an increase of cell size due to tetraploidization is not direct or automatic. Further construction and analyses of tetraploid mutants are in progress.

II. Biodiversity of leaf form

We are also interested in the biodiversity of wild plants. We found that *P. asiatica* is a cryptic allotetraploid from the molecular data, and pursued detailed molecular phylogenetic analyses of members of the subgenus *Plantago* of the genus *Plantago*. As a result, we found that most known species in this section are derived from inter-species hybridization. Surprisingly, whole species in some sections are found to be derived from inter-section hybridization, showing that subgenus *Plantago* is derived from extensive reticulate evolution (Ishikawa et al., 2009).

Publication List

〔Original papers〕

- Fujikura, U., Horiguchi, G., Ponce, M.R., Micol, J.L., and Tsukaya, H. (2009). Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana*. Plant J. *59*, 499–508.
- Horiguchi, G., Gonzalez, N., Beemster, G.T.S., Inzé, D., and Tsukaya, H. (2009). Impact of segmental chromosomal duplications on leaf size in the *grandifolia-D* mutants of *Arabidopsis thaliana*. Plant J. *60*, 122-133.
- Ishikawa, N., Yokoyama, J., and Tsukaya, H. (2009). Molecular evidence of reticulate evolution in the subgenus Plantago (Plantaginaceae). Amer. J. Bot. *96*, 1627–1635.
- Usami, T., Horiguchi, G., Yano, S. and Tsukaya, H. (2009). The more and smaller cells mutants of *Arabidopsis thaliana* identify novel roles for *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes in the control of heteroblasty. Development *136*, 955-964.

〔Review article〕

- Tsukaya, H. (2009). Size regulation of plant body via control of cell volume by endoreduplication. Reg. Plant Growth Dev. *44*, 135-141.

LABORATORY OF PHOTOENVIRONMENTAL BIOLOGY

Professor (Adjunct)
WATANABE, Masakatsu

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

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

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

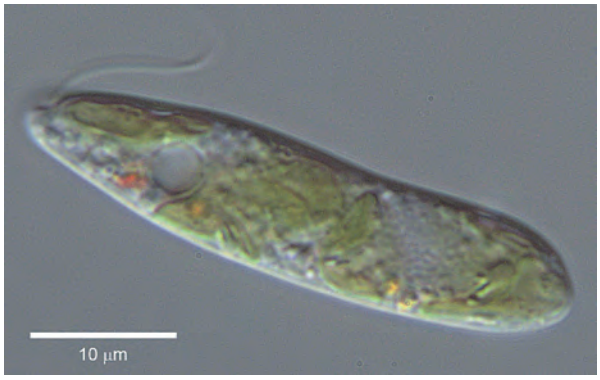


Figure 1. *Euglena gracilis*, a unicellular flagellate alga. It swims forward (to the left) by shaking the flagellum, the protruding whip-like structure. The flagellar motion is controlled by ultraviolet to blue light signals sensed by the photoreceptor molecules in the "real eye" located adjacently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

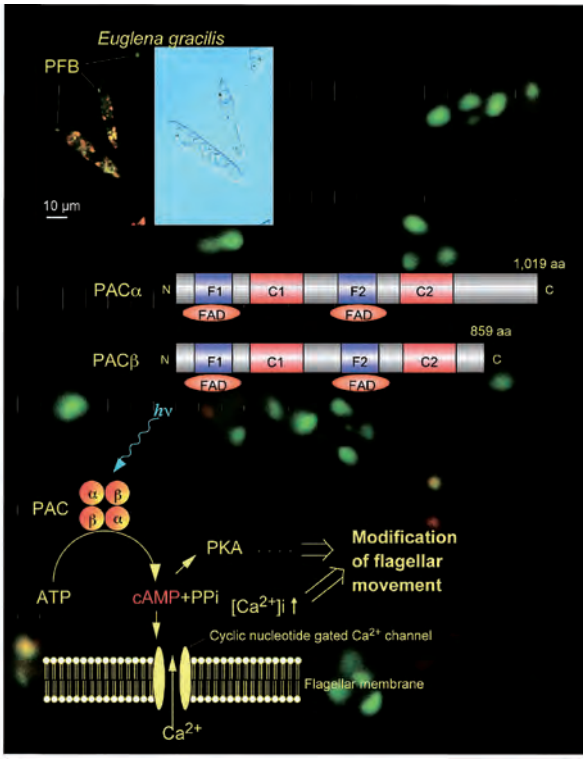


Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

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

II. Flagellar motions during algal phototactic steering

To understand the mechanism of blue-light-induced algal phototactic steering, we observed, using infrared high-speed video microscopy, light-triggered transitory flagellar motions in flagellate reproductive cells (swarmers) of a brown alga, *Scytosiphon lomentaria*, under primary helical swimming conditions before and during negative phototactic orientation to unilateral actinic light (Figure 3).

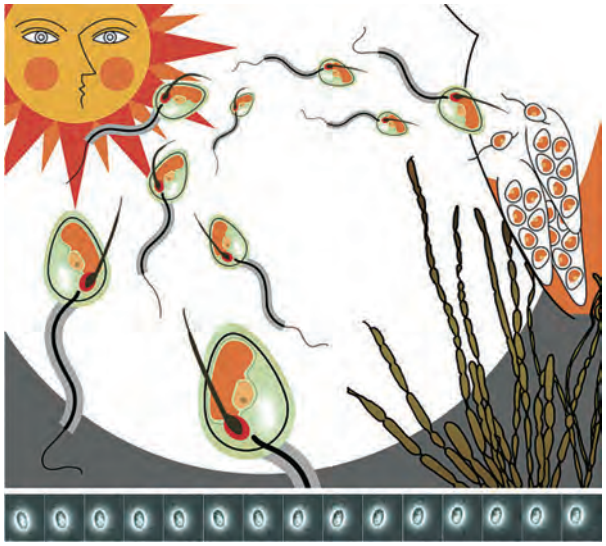


Figure 3. Photo-orientation of swarmer cells released from matured thalli of the brown alga, *Scytosiphon lomentaria*. Each swarmer has a hairy long anterior flagellum and a smooth short and autofluorescent posterior one. Cover illustration of Photochem. Photobiol. 86 (2) drawn by Hiroko Uchida

The posterior flagellum, which is autofluorescent and thought to be light-sensing, was passively dragged in the dark and exhibited one to several rapid lateral beats during orientation changes for phototactic steering (Figure 4). Notably, a brief cessation of anterior flagellar beating was occasionally observed concomitantly with rapid beats of the posterior flagellum. This behavior caused a pause in helical body rotation, which may contribute to the accuracy of phototactic steering (Figure 5). Thus, coordinated regulation of the movement of the two flagella plays a crucial role in phototactic steering.

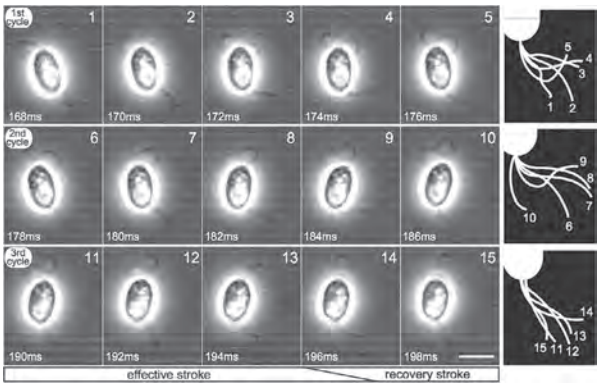


Figure 4. Rapid beats of posterior flagellum after onset of light stimulus observed at 2 ms intervals.

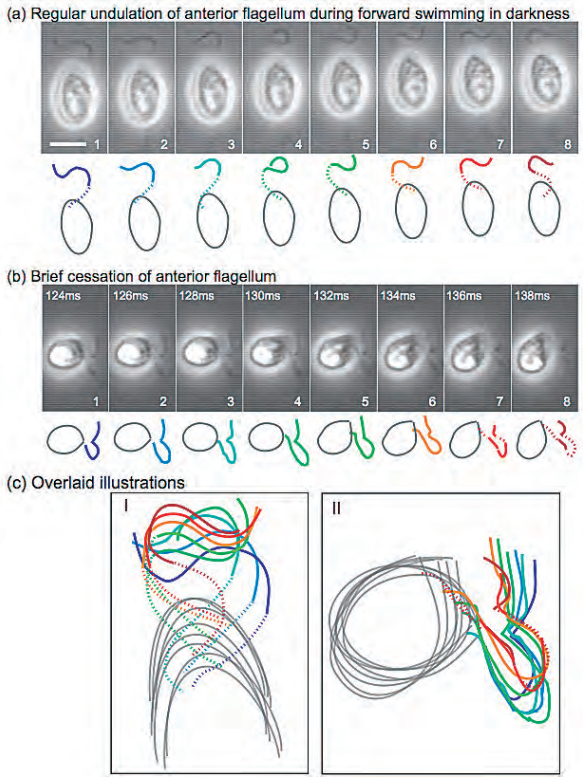


Figure 5. A brief cessation of anterior flagellum after onset of the light stimulus.

Publication List

〔Original paper〕

- Izawa, N., Suzuki, T., Watanabe, M., and Takeda, M. (2009). Characterization of arylalkylamine N-acetyltransferase (AANAT) activities and action spectrum for suppression in the band-legged cricket, *Dianemobius nigrofasciatus* (Orthoptera: Gryllidae). Comp. Biochem. Physiol. Part B *152*, 346–351.

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

- Matsunaga, S., Uchida, H., Iseki, M., Watanabe, M., and Murakami, A. Flagellar motions in phototactic steering in a brown algal swarmer. Photochem. Photobiol. 2009 Dec. 14.

DIVISION OF THEORETICAL BIOLOGY

Professor (Concurrent)
MOCHIZUKI, Atsushi

We are studying biological phenomena using mathematical models. This method gives us an integrative understanding of the behavior of complex systems in biology including gene regulatory networks.

Mathematical models are especially useful in understanding pattern formation in development. The study of the mechanisms responsible for morphological differences between species is an important research focus of current developmental biology.

I. Steepness of thermal gradient is essential to obtain a unified view of thermotaxis in *C. elegans*

One of the adaptive behaviors of animals in their environment is thermotaxis, by which they migrate toward a preferred temperature. This sensorimotor integration is accomplished by choosing one of two behaviors depending on the surrounding temperature, namely thermophilic or cryophilic movement. C. elegans exhibits thermotaxis and its migration behavior has been analyzed experimentally at both the population and individual levels. However, some experimental data are inconsistent especially for thermophilic movement, which is expected to be observed in lower than favorable temperatures. There are no experimental analyses that find thermophilic tendencies in the individual behavior of worms, despite multiple reports supporting thermophilic movement of the population. Although theoretical methods have been used to study thermotaxis of C. elegans, no mathematical model provides a consistent explanation for this discrepancy. This study was done by Dr. Nakazato in our group as a a collaboration work with Drs. Mori and Kuhara in Nagoya University.

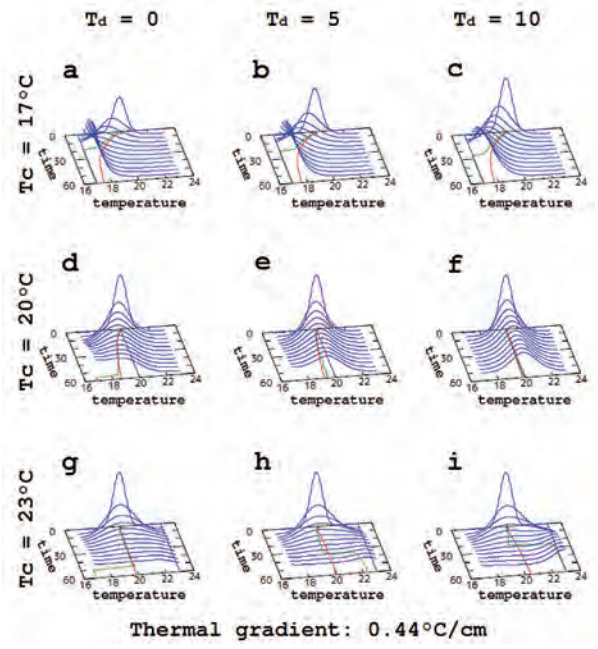


Figure 1. Thermophilic tendency in distribution behavior can be seen only if we adopt the parameter sets with a thermophilic bias. Sufficiently strong thermophilic bias is needed to observe thermophilic tendency in distribution behavior. Red, green and purple lines show the mean of population, the position of maximum peak and cultivated temperature respectively.

Here we develop a simple biased random walk model, which describes population behavior, but which is based on the results of individual assays. Our model can integrate all previous experiments without any contradiction. We regenerate all the population patterns reported in past studies and give a consistent explanation for the conflicting results. Our results suggest that thermophilic movement is observed, even in individual movements, when the thermal gradient is sufficiently slight. On the contrary, thermophilic movement disappears when the thermal gradient is too steep. The thermal gradient is thus essential for a comprehensive understanding of the experimental studies of thermotaxis in C. elegans. Our model provides insight into an integrative understanding of the neural activity and thermotactic behavior in C. elegans.

II. Mathematical modeling for gene expression of vertebrate segmentation

Segmentation in the vertebrate PSM (presomitic mesoderm) is established by a series of pattern formation through the dynamics of gene expression at different levels. Some downstream genes suppress the activity of upstream genes. The negative feedback seems to realize transient dynamics of patterning. We developed two mathematical models, where the negative-regulator genes are different. We found that the previously believed regulation model cannot explain the mutant expression patterns, but the newly proposed model can. The mathematical model gives an integrative understanding and a working hypothesis for a regulatory network system including many genes. This study was done by Dr. Saitou of our group at the RIKEN Advanced Science Institute in collaboration with Drs. Takada and Takahashi of NIBB.

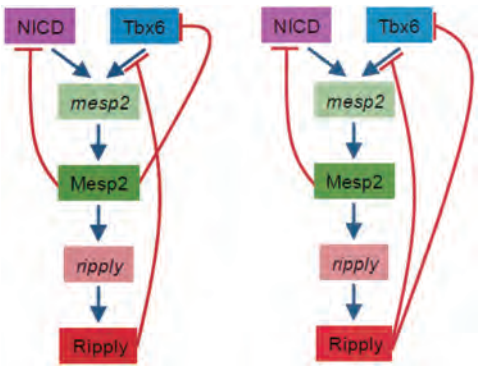


Figure 2. Two models for the regulations of segmentation genes. Left; Model A (Previously believed), Right; Model B (Our hypothesis).

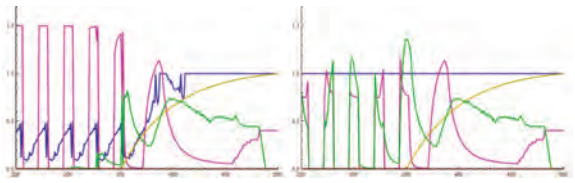


Figure 3. Dynamics of gene expressions for wild type are almost equivalent between the two models. Left; Model A (Previously believed), Right; Model B (Our hypothesis).

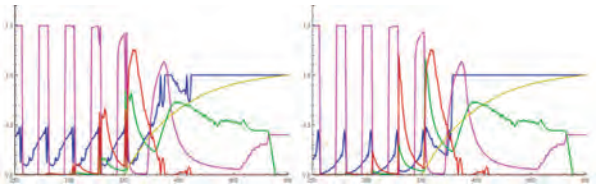


Figure 4. Dynamics of gene expressions for *Ripply* null-mutant are completely different between the two models. Left; Model A (Previously believed), Right; Model B (Our hypothesis). Only model B can explain the experimental results.

III. Structure of regulatory networks and diversity of gene expression patterns

The complexity of gene regulatory networks is considered responsible for the diversity of cells. Different types of cells, characterized by the expression patterns of genes, are produced in early development through the dynamics of gene activities based on the regulatory network. However, very little is known about the relationship between the structure of regulatory networks and the dynamics of gene activities.

In this study I introduce the new idea of "steady state compatibility," by which the diversity of possible gene activities can be determined from the topological structure of gene regulatory networks. The basic premise is very simple: the activity of a gene should be a function of the controlling genes. Thus a gene should always show unique expression activity if the activities of the controlling genes are unique. Based on this, the maximum possible diversity of steady states is determined using only information regarding regulatory linkages and without knowing the regulatory functions of genes.

Using the concept of "steady state compatibility," three general properties of the relationship between the topology of regulatory networks and the maximum number of steady states can be derived (Figure 2). (A) Cascade structures in regulatory networks do not increase the number of possible steady states (Fig. 2a). (B) Loop structures in networks are necessary to generate multiple steady states. The number of separated loops increases the maximum diversity of steady states (Fig. 2b). (C) Multiple loops that are connected by sharing the same genes do not increase the maximum diversity of steady states (Fig. 2c).

The method was applied to a gene regulatory network responsible for early development in a sea urchin species. A set of important genes responsible for generating diversities of gene activities was derived based on the concept of compatibility of steady states.

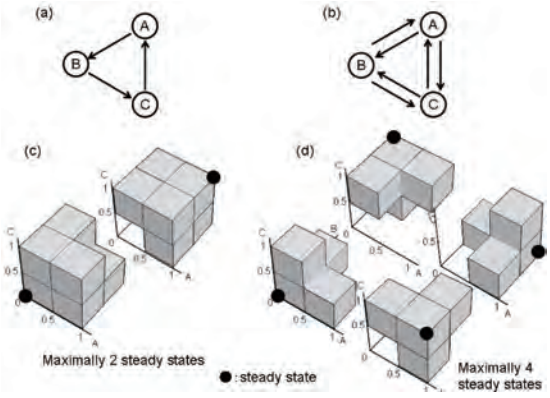


Figure 5. An intuitive explanation of "steady state compatibility". (a) An example of the regulatory links of a mono-directional loop with three genes. (b) Another example of the regulatory links of a bi-directional loop with three genes. (c) The shaded domains show the region where other steady states should not appear except for the original point (0,0,0) and (1,1,1) based on the network in (a). The network (a) has two steady states at maximum. (d) The network (b) determines the different shapes of the domains of no-steady-state except for the points (0,0,0), (0,1,1), (1,0,1) and (1,1,0). This network allows four steady states at maximum.

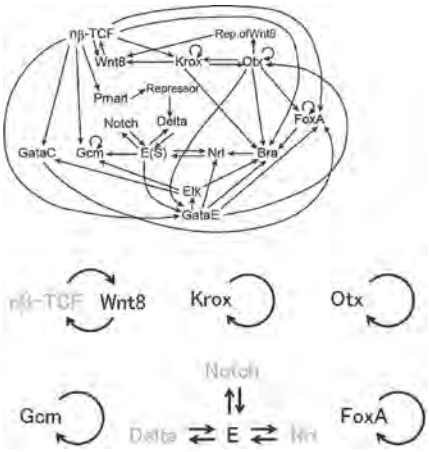


Figure 6. Analysis of an actual gene network responsible for the early development of a sea urchin species. (a) The network is simplified from the one of Fig. 3 in Davidson *et al*. (2002). The maximum diversity generated from this network is determined by the analysis as 64. (b) All of the "reduced observation point" ROP genes are derived. At least one of the ROPs should change its activities in the alternative steady states.

Publication List

〔Original paper〕

● Nakazato, K., and Mochizuki, A. (2009). Steepness of thermal gradient is essential to obtain a unified view of thermotaxis in C. elegans. J. theor. Biol. *260*, 56-65.

LABORATORY OF GENOME INFORMATICS

Assistant Professor
UCHIYAMA, Ikuo

Postdoctoral Fellow: *KAWAI, Mikihiko*

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

I. Microbial genome database for comparative analysis

The microbial genome database for comparative analysis (MBGD) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes from precomputed all-against-all similarity relationships using the DomClust algorithm (see Section II below). By means of this algorithm, MBGD not only provides comprehensive orthologous groups among the latest genomic data available, but also allows users to create their own ortholog groups on the fly using a specified set of organisms. The latter feature is especially useful when the user's interest is focused on some taxonomically related organisms.

This year, we have developed the following advanced functionalities: 1) enhanced assignment of functional annotation, including external database links to each orthologous group, 2) an interface for choosing a set of genomes based on phenotypic properties (Figure1), 3) the addition of more eukaryotic microbial genomes (fungi and protists) and some higher eukaryotes as references, 4) enhancement of the MyMBGD mode, which allows users to add their own genomes to MBGD and now accepts raw genomic sequences without any annotation (in such cases it runs a gene-finding procedure before identifying the orthologs). Some analysis functions, such as the function to find orthologs with similar phylogenetic patterns, have also been improved. The database now contains around 1000 published genomes including 16 eukaryotic microbes and 4 multicellular organisms.

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

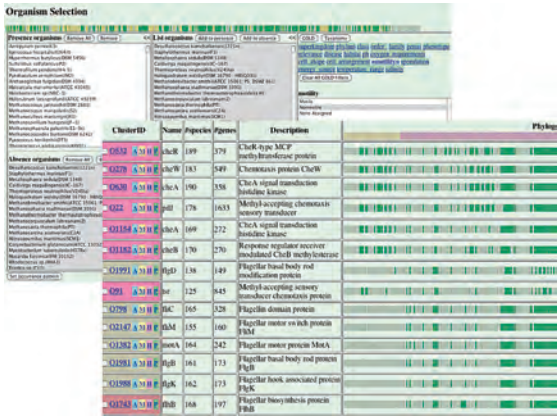


Figure 1. The interface for organism selection in MBGD where a phylogenetic pattern related to "motility" is specified (top-left) and the result of the pattern search (bottom-right).

II. Enhancement of the algorithm for identifying orthologous groups among multiple genomes

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

We are continuing to improve the algorithm. Partial taxonomic information can be incorporated into the DomClust algorithm by specifying ingroup/outgroup for each input genome. The resulting table has a nested structure when a duplication event occurs within the ingroup lineage. We are also trying to modify the algorithm for utilizing metagenomic analysis. In addition, to further improve scalability for comparison of thousands of genomic sequences, we are now developing an efficient method to update the clustering result incrementally.

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

Horizontal gene transfers (HGT) have played a significant role in prokaryotic genome evolution, and the genes constituting a prokaryotic genome appear to be divided into two classes: a "core gene pool" that comprises intrinsic genes encoding the proteins of basic cellular functions, and a "flexible gene pool" that comprises HGT-acquired genes encoding proteins which function under particular conditions. The identification of the set of intrinsically conserved genes, or the genomic core, among a taxonomic group is crucial not only for establishing the identity of each taxonomic group, but also for understanding prokaryotic diversity and evolution. We consider the core structure of related genomes as a set of sufficiently long segments in

which gene orders are conserved among multiple genomes so that they are likely to have been inherited mainly through vertical transfer. We developed a method for aligning conserved regions of multiple genomes, which finds the order of pre-identified orthologous groups that retains to the greatest possible extent the conserved gene orders.

The program, named CoreAligner, was successfully applied to the genome sequences of two major bacterial families, *Bacillaceae* and *Enterobacteriaceae*, for identifying the core structures comprising 1438 and 2125 orthologous groups, respectively. We are now expanding our analysis to more diverged bacterial families to examine generality of our approach. We are also developing an enhanced algorithm that can incorporate phylogenetic relationships among input genomes.

IV. Development of a workbench for comparative genomics

We are developing a comparative genomics workbench named RECOG (Research Environment for COmparative Genomics), which aims to extend the current MBGD system by incorporating more advanced analysis functionalities including phylogenetic pattern analysis, the ingroup/outgroup distinction in ortholog grouping and the core structure extraction among related genomes. The entire RECOG system employs client-server architecture: the server program is based on the MBGD server and contains the database construction protocol used in MBGD so that users can install the server on their local machines to analyze their own genomic data, whereas the client program is a Java application that runs on a local machine by receiving data from any available RECOG server including the public MBGD server. The main window of the RECOG client consists of a taxonomic tree viewer (left), an ortholog table/phylogenetic pattern map viewer (center) and a gene information viewer (right). Users can choose a set of genomes using the taxonomic tree viewer and run the DomClust program to identify orthologous groups. The result is displayed in the ortholog table viewer, where users can see the entire picture of the phylogenetic pattern map as well as the full details of the ortholog table using semantic zooming functionality.

This year, we added several new functions to the RECOG system to enhance its usability for comparative analysis. One of the new features is gene property analysis, in which users can define property values for each gene and compare these values among orthologous genes on the ortholog table viewer (Figure 2).

Figure 2. The phylogenetic pattern viewer comparing ten *H. pylori* strains (see section V), where each cell is colored according to G+C content at the third codon positions using the gene property analysis functionality.

V. Comparative genomics of *Helicobacter pylori*

Helicobacter pylori is a major pathogen in human gastric cancer and it is known that East Asian strains of *H. pylori* have a more toxic CagA protein, a major virulence factor, than Western strains. In collaboration with Dr. Kobayashi (Univ. Tokyo) and other researchers, we have determined the complete genomic sequences of four *H. pylori* strains isolated from Japanese patients and compared them with published *H. pylori* genomes. Although all four genomes are clearly classified into the East Asian group, they exhibit substantial polymorphisms in genomic structure (Figure 3). We are trying to identify genomic features specific to East Asian strains and infer evolutionary processes and mechanisms that are related to the evolution of *H. pylori*.

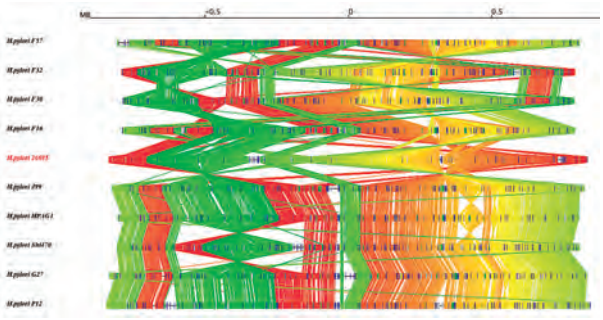


Figure 3. Comparative genome map among *H. pylori* strains. Genomic core structure is identified by CoreAligner and orthologous genes are connected with lines. Colors are assigned according to the gene order in one strain (26695).

Publication List

〔Original papers〕

- Baba, T., Kuwahara-Arai, K., Uchiyama, I., Takeuchi, F., Ito, T., and Hiramatsu, K. (2009). Complete genome sequence determination of a *Macrococcus caseolyticus* strain JSCS5402 reflecting the ancestral genome of the human pathogenic staphylococci. J. Bacteriol. *191*, 1180-1190.
- Watanabe, S., Ito, T., Sasaki, T., Li, S., Uchiyama, I., Kishii, K., Kikuchi, K., Skov, R.L., and Hiramatsu, K. (2009). Genetic diversity of staphylocoagulase genes (coa): insight into the evolution of variable chromosomal virulence factors in *Staphylococcus aureus*. PLoS One *4*, e5714.

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

- Uchiyama, I., Higuchi, T., and Kawai, M. MBGD update 2010: toward a comprehensive resource for exploring microbial genome diversity. Nucleic Acids Res. 2009, Nov. 11.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

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

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

I. Initial step for left-right asymmetry

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

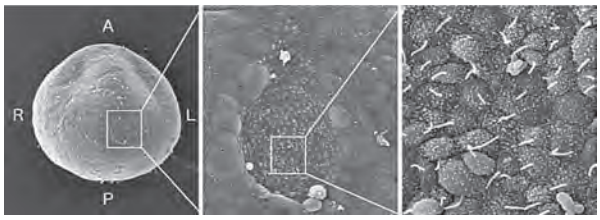


Figure 1. Left: ventral view of a 7.5-day mouse embryo. Middle: the node. Right: node cilia.

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

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

II. Imaging technologies

Long-term live imaging of large specimens, such as embryos, is very useful in developmental biology but technically challenging, mainly because of phototoxicity and the limitations of deep imaging. The Digital Scanned Light-sheet Microscope (DSLM, Figure 2) developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL) is extremely suitable for this purpose, and we have introduced a DSLM set and applied it to visualize cell movements in intact mouse embryos at gastrulating stages.

Additionally, we support researchers who are interested in using our DSLM and two-photon microscope (for example, Hashimoto et al. Nat. Cell Biol. 12, 170-186, 2010). Several collaborative projects are in progress.

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

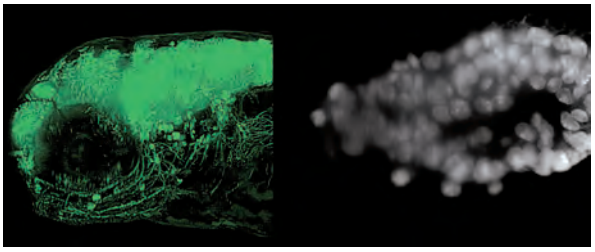


Figure 3. Images taken with DSLM. Left: optical section of a 6-day mouse embryo with fluorescent nuclei. Right: a juvenile zebrafish head expressing GFP in neurons.

Publication List

〔Review article〕

● Nonaka, S. (2009). Modification of Mouse Nodal Flow by Applying Artificial Flow. In Methods in Cell Biology, S. King, and G. Pazour, eds. (Academic Press), pp. 287-297.

KAMADA GROUP

Assistant Professor: *KAMADA,Yoshiaki*

Cell growth (increase in cell mass) and proliferation (increase in cell number) are highly linked with the cell's perception of its nutritional environment. Tor (target of rapamycin) protein belongs to a family of phosphatidylinositol kinase-like protein kinases and plays a central role in controlling cell growth. Specifically, Tor signaling couples nutrient signals to various growth-related processes. Tor protein forms distinct Tor complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as translation initiation, ribosome biogenesis, and autophagy. On the other hand, TORC2, whose function is insensitive to rapamycin, is responsible for actin organization and cell integrity.

The aim of this research group is to reveal the molecular mechanisms of how the Tor pathway regulates each phenomenon, and how Tor receives nutrient signals. We have been studying Tor signaling in the budding yeast, *Saccharomyces cerevisiae*, and have found three novel branches of the TOR signaling pathway.

I. TORC1 is involved in the cell cycle at G2/M transition

It is well known that inhibition of protein synthesis causes cell growth and promotes cell cycle arrest at G1 (G0). Rapamycin, a TORC1 inhibitor has the same effect via inactivating TORC1. 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*, which encodes an essential component of TORC1. We found that this mutant, as well as yeast cells treated with rapamycin, exhibits mitotic delay with prolonged G2. We further demonstrated that this G2-arrest phenotype is due to mislocalization and resultant inactivation of Cdc5, the yeast polo-kinase. These results suggest that TORC1 mediates G2/M transition via regulating polo-kinase.

II. TORC1 regulates autophagy via Atg1 kinase complex

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 Atg13. Although Atg13 is phosphorylated in a TORC1-dependent manner under nutrient-replete conditions, it is immediately dephosphorylated in response to starvation or rapamycin treatment. Dephosphorylated Atg13 binds to Atg1 which allows Atg17, Atg29, and Atg31 to associate with Atg1-Atg13 to form Atg1 complex. Atg1 complex formation triggers autophagy at least in two ways. First, through the recruitment of Atg proteins to the pre-autophagosomal structure (PAS), the putative site for autophagosome formation. Second, Atg1 kinase activity is enhanced by Atg1 complex formation. We assume that Atg1 phosphorylates its substrate(s) at the PAS to trigger a downstream event in autophagy. (Figure 1)

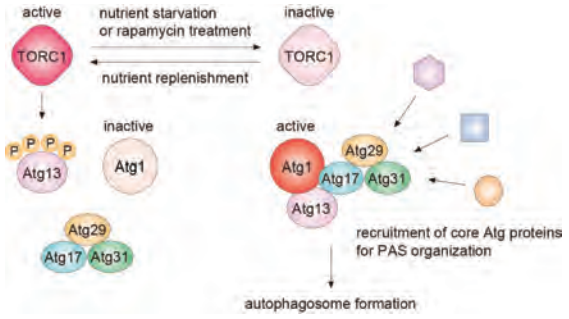


Figure 1. A schematic model of regulation of Atg1 complex by TORC1. When TORC1 is inactivated following nutrient starvation, Atg13 is dephosphorylated. This allows the formation of Atg1 complex among Atg1 and its regulators, followed by the upregulation of the Atg1 kinase activity and assembly of other Atg proteins to the PAS to initiate autophagosome formation.

III. TORC2 phosphorylates Ypk2 kinase to control actin organization

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

Publication List

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

● Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., and Ohsumi, Y. Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol. Cell Biol. 2009 Dec 7.

〔Review article〕

● Nakatogawa, H., Suzuki, K., Kamada, Y., and Ohsumi, Y. (2009). Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol. *10*, 458-467.

OHNO GROUP

Assistant Professor:	*OHNO, Kaoru*
Technical Assistants:	*HARA, Ikuyo*
	FUKAZAWA, Mitsue
	INAGAKI, Masako

The aim of this laboratory is to research the 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 oe 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 hormonesnare assumed to be the physiological counterpart of LH and FSH in mammals. Some gonadotropic hormones, such as egg development neurosecretory hormone of the mosquito, egg-laying hormone of the sea hare, and androgenic gland hormone of the terrestrial isopod, have been found iw 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 GSSdreferred to aminodacid 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).

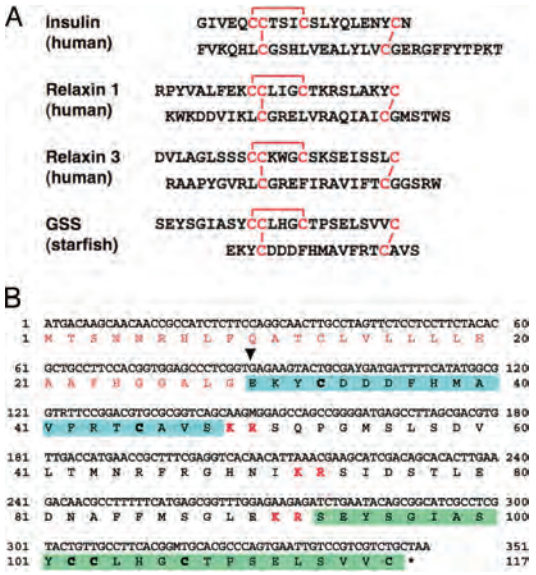


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

Publication List

〔Original paper〕

- Mita, M., Yoshikuni, M., Ohno, K., Shibata, Y., Paul-Prasanth, B., Pitchayawasin, S., Isobe, M., and Nagahama, Y. (2009). A relaxin-like peptide purified from radial nerves induces oocyte maturation and ovulation in the starfish, *Asterina pectinifera*. Proc. Natl. Acad. Sci. USA *106*, 9507-9512.

TERADA GROUP

Assistant Professor: *TERADA, Rie*
Postdoctoral Fellow: *SHIMATANI, Zenpei*
Technical Assistants: *ASAO, Hisayo*
*SHIMATANI, Zenpei**
MORITOH, Satoru

With the goal of developing an effective molecular breeding method and progressing in our continuing gene function study in rice (*Oryza sativa* L.), an important staple food, we are developing a new technology of gene targeting (GT) mediated by homologous recombination (HR). We have succeeded in gene targeting of *Waxy* based on a strong positive-negative (PN) selection in the cell where HR at the *Waxy* locus took place. Because our GT procedure is applicable to modify various genome regions we have been able to target 15 individual rice genes using the published sequence information. We have also succeeded in making various modifications to the target genes including gene knock-out as well as knock-in targeting of the coding sequence of *GUS* to the targeted gene promoter for analysis of its natural activity. In addition the point mutations on the vector for GT were effectively integrated into the targeted gene locus in *Alcohol dehydrogenase* gene 2 (*Adh2*), and a re-activation system of targeted-*waxy* by Cre-*loxP* recombination (Terada et al., Plant Biotech., in press) was achieved, similar to that used in mouse conditional GT. These results suggest lots of possibilities for creation of ideal mutants for GT-mediated molecular breeding and detailed gene function studies.

I. Generation of blast fungus resistant rice based on effective GT

OsRac1, a homolog of mammalian Rac GTPase, plays an important role in the defense response of plants. Amino acid substitution of the 19th glycine to valine (G19V) alters OsRac1 to be constitutively active (CA) by elimination of its GTPase activity. Increased resistance to blast fungus was detected in random transgenic rice of CA-*OsRac1* driven by 35S promoter. To examine whether the endogenous *OsRac1* shows the same effect or not we generated CA-*OsRac1* using our rice GT procedure. We constructed targeting vectors to induce the G19V substitution through a single point mutation of G to T in the first exon of *OsRac1*. Elimination of the positive marker, *hygromycin phospho transferase* (*hpt*), from targeted *OsRac1* using our Cre-*loxP* system created CA-*OsRac1* at the natural gene locus. Two targeting vectors, pRac1A and pRac1B, were constructed to have homologous sequences of *OsRac1* covering slightly different regions each other in order to insert the *hpt* marker flanked by the *loxP* sites ('floxed') into the first and third intron, respectively (Figure 1). After the G to T substitution by GT the *hpt* is eliminated by the Cre-*loxP* system. A single *loxP* remains in the first or third intron, however, these are expected to splice out. We have succeeded in GT of *OsRac1* using both vectors (Table 1). About 14% of PN selected calli were successfully targeted, giving us the highest efficiency of GT ever obtained. Interestingly in addition to the expected true GT (TGT), one-sided invasion (OSI; HR is caused only at 5' or 3' end of integrated DNA fragment) was frequently detected during GT using either vectors (Table 1). We selected 16 callus lines showing TGT and applied Cre mediated *hpt* elimination. Finally 10 plants regenerated from 4 *hpt* eliminated marker-free lines were grown all of which revealed characteristic semi-dwarf phenotype. Precise G to T substitution and *hpt* elimination in the heterozygous form was confirmed by sequence analyses of obtained plant DNA. Because fungus resistant phenotype is dominant, obtained targeted CA-*OsRac1* plants are under examination for resistance to blast fungus.

This work is a collaboration with Professor Ko Shimamoto in the Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology (NIST) and supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan Grant-in-Aid for Scientific Research (S) (No. 19108005 to K.S.)

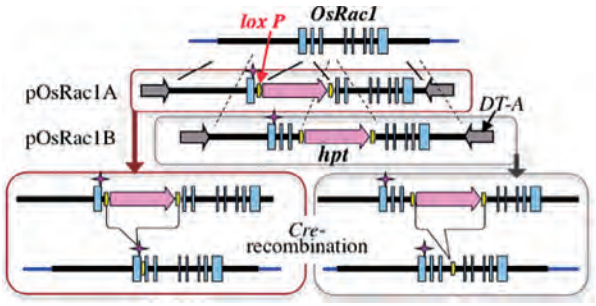


Figure 1. Strategy for creation of CA-*OsRac1*. Star indicates point mutation of G to T.

Targeted gene	PN selected Calli	OSI		TGT	Ratio of TGT/PN (%)
		5'only	3'only	5'+3'	
Waxy	638	0	0	6	0.94
OsRac1 by pRac1A	94	10	37	5	5.5
OsRac1 by pRac1B	80	4	4	11	13.6

Table I. Gene targeting of *OsRac1*

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

HOSHINO GROUP

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

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

I. The morning glories

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

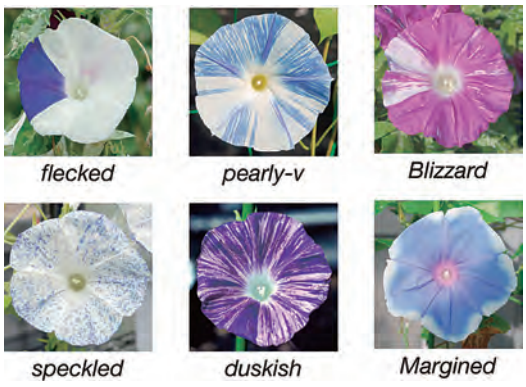


Figure 1. Flower phenotypes of Japanese morning glories.

II. Flower pigmentation patterns

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

III. BioResource of morning glories

NIBB is the sub-center for National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* has been one of the most popular floricultural plants since the late Edo era in Japan. It has extensive history of genetic studies and also has many advantages as a model plant; simple genome, large number of mutant lines, and efficient self-pollination. Our collection increased to 200 lines and 100,000 clones by the end of 2009. A web site to integrate information on these resources is under construction (Figure 2).

Figure 2. NBRP morning glory web site.

Publication List

〔Original papers〕

- Hoshino, A., Park, K.I., and Iida, S. (2009). Identification of *r* mutations conferring white flowers in the Japanese morning glory, *Ipomoea nil*. J. Plant Research *122*, 215-222.
- Shimatani, Z., Takagi, K., Eun, C.H., Maekawa, M., Takahara, H., Hoshino, A., Qian, Q., Terada, R., Johzuka-Hisatomi, Y., Iida, S., and Tsugane, K. (2009). Characterization of autonomous *Dart1* transposons belonging to the *hAT* superfamily in rice. Mol. Gen. Genomics *281*, 329-344.

TSUGANE GROUP

Assistant Professor: *TSUGANE, Kazuo*

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

Gene tagging is an important tool for understanding gene functions. We have constructed mutant rice lines using DNA transposons in order to achieve functional genomics analysis in rice, a model plant for monocots and cereals.

I. An active DNA transposon in rice

The mutable allele *virescent* (or *pale-yellow-leaf variegated, pyl-v*) which displays leaf variegation was caused by the integration of the 607-bp non-autonomous element *nDart1-0* belonging to the *hAT* superfamily into the *OsClpP5* gene encoding the chloroplast protease. In the mutable *pyl-v* allele, somatic excision of *nDart1-0* from *OsClpP5* in the presence of an active autonomous element, *aDart*, results in the pyl-v leaf variegation phenotype, a dark-green sector consisting of somatically reverted cells, due to *nDart1-0* excision, on a pale-yellow background comprising of cells having *nDart1-0* inserted into *OsClpP5* in the homozygous condition. Plants containing the *pyl-v* allele without an active *aDart* element display pale-yellow leaves without variegation; this has been termed as the pale-yellow leaf-stable (pyl-stb) phenotype. In the sequenced Nipponbare genome containing no active *aDart* elements, among the 53 inactive *iDart1* elements, there are 38 putative autonomous *iDart1* elements. Because their putative transposase genes carry no apparent nonsense or frameshift mutations, they are thought to be silenced epigenetically. Therefore we felt it highly likely that an active *aDart* element would be similar in structure to one of these 38 *iDart1* elements. Using map-based cloning, we found that *aDart* in the mutable *pyl-v* plant coincides with one of the 38 *iDart1* elements, *iDart1-27*, residing on chromosome 6 in Nipponbare and that the transcripts of the accumulated transposase gene in the *pyl-v* leaves are predominantly from *Dart1-27*. Two additional smaller transcripts were detected in *pyl-v* (Figure. 1). The major transcripts detected in *pyl-stb* and Nipponbare were derived from non-*Dart1-27* elements, implying that the residual expression of other *Dart1* elements in both *pyl-stb* and Nipponbare would be too weak to act on the *nDart1-0* at *OsClpP5* and lead to their excision even though some of these transcripts might encode an active transposase. While the longest (L) transcripts in *pyl-v* are the *Dart1-27* transcripts having intron 1 at 5'-UTR spliced, the middle (M) and the shortest (S) transcripts were mixtures that had one or two additional introns spliced, respectively, although the exact splicing sites were often different. It is noteworthy that all the shorter transcripts characterized in *pyl-v* were derived from *Dart1-27*, indicating that some of the abundantly expressed transcripts must have undergone further splicing in rice. These findings should facilitate the development of an efficient gene-tagging system in rice and shed light on epigenetic regulatory and evolutionary aspects of autonomous elements in the *nDart*/*aDart* system.

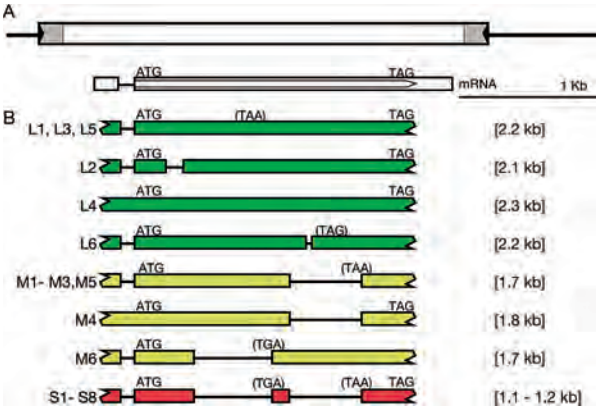


Figure 1 Transcripts of the *Dart1* transposase genes detected in rice. **(A)** Structure of *Dart1-27* in the *pyl-v* genome. **(B)** Schematic representation of the alternatively spliced transposase transcripts observed in rice plants. Three different sized transcripts (L, M, and S) were observed, and their splicing patterns are categorized.

II. Reverse genetic analysis of rice genes

Among active rice DNA transposons, *nDart1-0* and its relatives appear to be more suitable than the others for transposon tagging in rice because (1) their transposition can be controlled under natural growth conditions, *i.e.*, the transposition of *nDart1-0* can be induced by crossing with a line containing an active *aDart* element and stabilized by segregating *aDart*, and (2) *nDart1-0* and its relatives are often found at GC-rich regions in the genome and tend to integrate into promoter proximal genic regions. Because we have also obtained dominant and semi-dominant mutants (Figure 2), *nDart1*-promoted mutant lines can contribute to functional genomics in rice.

Figure 2. Semi-dominant *bushy dwarf tillers* mutant.

Publication List

〔Original paper〕

● Shimatani, Z., Takagi, K., Eun, C.H., Maekawa, M., Takahara, H., Hoshino, A., Qian, Q., Terada, R., Johzuka-Hisatomi, Y., Iida, S., and Tsugane, K. (2009). Characterization of autonomous *Dart1* transposons belonging to the *hAT* superfamily in rice. Mol. Gen. Genomics *281*, 329-344.

RESEARCH SUPPORT FACILITIES

Head
TAKADA, Shinji

● ***Large Spectrograph Laboratory***

Professor (Adjunct):	*WATANABE, Masakatsu*
Technical Staff:	*HIGASHI, Sho-ichi*
Technical Assistants:	*ENDOU, Seiichiro*
	ICHIKAWA, Chiaki
Secretary	*ISHIKAWA, Azusa*

● ***Tissue and Cell Culture Laboratory***

Assistant Professor:	*HAMADA, Yoshio*
Technical Assistant:	*TAKESHITA, Miyako*

● ***Computer Laboratory***

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

● ***Plant Culture Laboratory***

Technical Staff:	*NANBA, Chieko*
Technical Assistant:	*SUZUKI, Keiko*

1. The Large Spectrograph Laboratory

This laboratory provides, for cooperative use, the largest spectrograph in the world, the Okazaki Large Spectrograph (OLS), dedicated to action spectroscopical studies of various light-controlled biological processes. The spectrograph runs on a 30kW Xenon arc lamp and has a compound grating composed of 36 smaller individual gratings. It projects a spectrum of a wavelength range from 250nm (ultraviolet) to 1,000nm (infrared) onto its focal curve of 10m in length. The fluence rate (intensity) of the monochromatic light at each wavelength is more than twice as much as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe *et al*., Photochem. Photobiol. *36*, 491-498, 1982).

Figure 1. The Large Spectrograph

The NIBB Cooperative Research Program for the Use of the OLS supports about 20 projects every year conducted by visiting scientists, including foreign researchers, as well as those in the Institute.

Action spectroscopical studies for various regulatory and damaging actions of light on living organisms, biological molecules, and artificial organic molecules have been conducted (cf. Watanabe, M. *In* "CRC Handbook of Organic Photochemistry and Photobiology, 2nd ed.". pp. 115-1~115-16, 2004).

An advanced irradiation system composed of CW lasers (364nm, 390-410nm, 440-460nm, 532nm, 655nm, 752nm) and uniform-fluence-rate irradiation optics interconnected by optical fibers was constructed in 2003. An advanced observation system for cellular and intracellular photobiological responses utilizing a two-photon microscope (FV300-Ix71-TP with a MaiTai laser) and a microbial photomovement analyzer (WinTrack2000/Ecotox) was also introduced.

2. Tissue and Cell Culture Laboratory

Various types of equipment for tissue and cell culture are provided. This laboratory is equipped with safety rooms which satisfy the P2/P3 physical containment level. This facility is routinely used for DNA recombination experiments.

3. Computer Laboratory

The computer laboratory maintains several computers to provide computation resources and the means of electronic communication within NIBB. Our main computer system, the Biological Information Analysis System (BIAS), consists of a shared memory parallel computer (SGI Altix 350; 8CPU, 48GB memory) with a disk array storage system (D-RAID; 1.6TB×10), a high-performance cluster system (DELL PowerEdge 1850; 2CPU×(16+1) nodes), and a data visualization terminal (DELL Precision 370). 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.

The computer laboratory also provides network communication services. Most of the PCs in each laboratory, as well as all of the above-mentioned service machines, are connected to each other by a local area network (LAN), which is linked to the high performance multimedia backbone network of the Okazaki National Research Institute (ORION). 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 contains the NIBB home page (http://www.nibb.ac.jp/).

4. Plant Culture Laboratory

Plant culture laboratory consist of biotron, plant cell culture facility, and experimental farm. Biotron contains a large number of culture boxes and a limited number of rooms with environmental control for plant culture. In some of these facilities and rooms, experiments can be carried out at the P1

physical containment level under extraordinary environments such as strong light intensity, low or high temperatures, etc. Plant cell culture facility is equipped with autotrophic and heterotrophic culture devices and equipment for experimental cultures of plant and microbial cells. Facilities for the preparation of plant cell cultures, including an aseptic room with clean benches, are also provided. Experimental farm consists of two 20 m^2 glass-houses with precise temperature and humidity control, three green houses at the P1 physical containment level, a small farm, and two greenhouses (45 and 88 m^2) with automatic sprinklers. The laboratory also includes a building with storage and work space.

Facilities for the preparation of plant cell cultures, including an aseptic room with clean benches, are also provided.

Publication List on OLS Collaboration

〔**Original papers**〕

- Izawa, N., Suzuki, T., Watanabe, M., and Takeda, M. (2009). Characterization of arylalkylamine *N*-acetyltransferase (AANAT) activities and action spectrum for suppression in the band-legged cricket, *Dianemobius nigrofasciatus* (Orthoptera: Gryllidae). Comp. Biochem. Physiol. B. *152*, 346–351.
- Suzuki, T., Izawa, N., Takeshima, T., Watanabe, M., and Takeda, M. (2009). Action spectrum for the suppression of arylalkylamine *N*-acetyltransferase activity in the two-spotted spider mite *Tetranychus urticae*. Photochem. Pyotobiol. *85*, 214-219.
- Suzuki, T., Watanabe, M., and Takeda, M. (2009). UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. J. Insect Physiol. *55*, 649-654.

CENTER FOR TRANSGENIC ANIMALS AND PLANTS

Head
IGUCHI, Taisen

Associate Professors:	*WATANABE, Eiji*
	SASAOKA, Toshikuni
	TANAKA, Minoru
Technical Staff:	*HAYASHI, Kohji*
	NOGUCHI, Yuji
Postdoctoral Fellows:	*YAMANAKA, Megumi*
	WATANABE, Kaori
Technical Assistants:	*KAWAMURA, Motofumi*
	INADA, Yosuke
	KAJIWARA, Yuya
	ICHIKAWA, Yoko
	TAKAGI, Yukari

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

The NIBB Center for Transgenic Animals and Plants (CTAP) was established in April 1998 to support research using transgenic and gene targeting techniques at NIBB. The CTAP is managed by the head (professor, a concurrent post) and three associate professors.

Figure 1. The new center facility for transgenic animals in the Yamate area

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 CTAP 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 and plants.
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 CTAP building in the Yamate area strengthened research activities using genetically altered organisms. The building has five floors and a total floor space of 2,500 m^2 in which we can generate, breed, store and analyze transgenic, gene targeting and mutant mice under SPF conditions. The mouse housing area was constructed based on a barrier system. This building is also equipped with breeding areas for transgenic small fish, birds and insects.

Figure 2. Liquid nitrogen storage equipment for cryopreservation

In 2009 (from January 1 to December 31) 4,639 mice and 1,107 fertilized eggs were brought into the CTAP in the Yamate area, and 38,835 mice (including pups bred in the facility) and 139 fertilized eggs were taken out.

A number of strains of genetically altered mice from outside the CTAP 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. At March 2009, we expanded the facility which includes areas for breeding, behavioral tests and transgenic studies using various kinds of recombinant viruses. In 2009 (from January 1 to December 31) 44 mice were brought into the CTAP in the Myodaiji area, and 585 mice (including pups bred in the facility) were taken out.

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

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

The first floor of the CTAP 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). For researchers who need fish, 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, five or more mutant lines and ten or more transgenic lines of medaka and zebrafish are maintained in our facility. In addition to the rooms mentioned above, another room is available for insects. All the rooms are qualified to meet the criteria for transgenic animals, allowing researchers to generate and maintain these important research tools.

In 2009 (from January 1 to December 31), 4,461 medaka and zebrafish (180 eggs, 1,230 embryos and 3,051 adults) were brought to the facility and 91,786 medaka and zebrafish (89,710 fertilized eggs, 1,070 embryos and 1,006 adults, including animals bred in the facility) were taken out. In the laboratory for chick embryos 7,925 fertilized chicken eggs were brought in and 23 fertilized eggs and 44 chicken embryos were taken out. 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, induced mutants and transgenic lines and training personnel regarding fish maintenance. As the result of the NBRP project, medaka transgenic strains have been successfully generated in the CTAP, which allows inducing the gene of interest by heat treatment in combination with a Cre/loxP system.

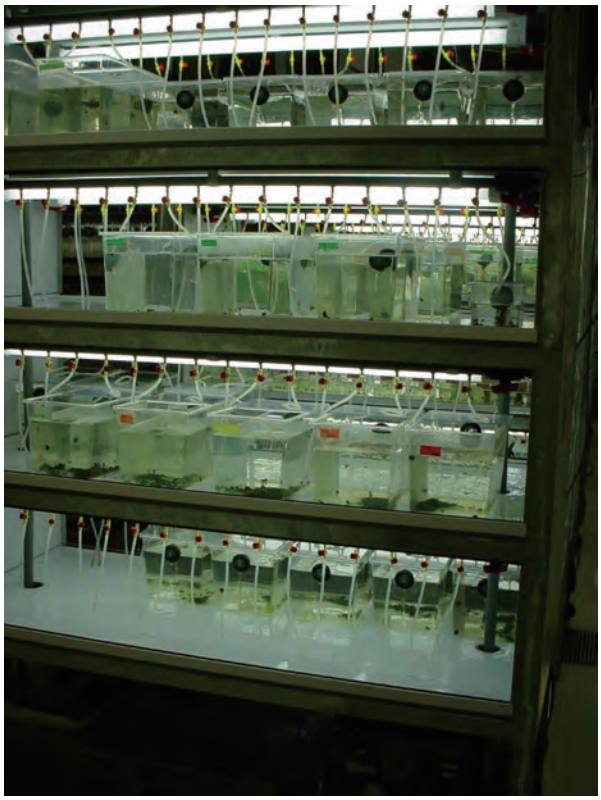


Figure 4. Breeding equipment for small fish

III. Academic activities

The associate professors of this center - E. Watanabe, T. Sasaoka and M. Tanaka - are the principal investigators of the Laboratory of Neurophysiology, the Laboratory of Neurochemistry and the Laboratory of Molecular Genetics for Reproduction, respectively. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. The Laboratory of Neurochemistry is studying the physiological role of the dopaminergic system using genetically altered mice. The Laboratory of 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 academic activities of each laboratory.

RESEARCH CENTER FOR INTEGRATIVE AND COMPUTATIONAL BIOLOGY

Head
HASEBE, Mitsuyasu

Professor (Concurrent): *MOCHIZUKI, Atsushi*

The aims of the research center for integrative and computational biology are (1) investigating the fundamental principles of various biological phenomena based on the integration of computational science and biology; (2) establishing new methodologies for integrative biology; and (3) providing new technology and knowledge to researchers. Our ultimate goal is to establish a new bioscience that includes methods originally utilized in different fields: informatics, mathematics, and biology.

I. Research activity

The research center for integrative and computational biology was founded in 2001 during a time of rapid progress in modern biology. The success of world wide genome projects has provided a huge amount of new information on genes, leading to expectations of advances in the development of new medicines for intractable diseases, the exploitation of new cultivated plants resistant to noxious insects, and so on. In addition, it is the object of present-day biology to research higher-order phenomena that are made up of complex interactions between many genes. To grapple with these challenges, it is necessary to decipher huge amounts of gene information and to reveal the fundamentals of the biological behavior of cells and organisms.

Mathematical and computational sciences have strong capacities for dealing with these challenges. Computational methods make it possible to process ever-increasing amounts of data. Hypothetical experiments (including the evolution of past organisms) based on mathematical or computational models make it possible to consider conditions which are impossible in real experiments. We continue to research higher-order phenomena in biology using mathematical and computational methods, as well as developing new methodologies for studying complex phenomena.

Figure 1. The laboratory room for computational studies

For example, integrative methods are especially important for understanding pattern formation in development. Morphological differences between species are an important research focus of current developmental biology. What is the mechanism responsible for the difference of morphogenesis between species? Theoretical studies are useful in identifying candidates for cell or gene interaction that are likely to be responsible for the systems in real organisms. This method gives us an integrative understanding of the behavior of complex systems in biology, including gene regulatory networks.

II. Collaborative activity

Interaction between researchers is essential to make the best use of mathematical and computational methods for experimental biology. Theoretical methods provide testable predictions which the experimental biologists are able to investigate before turning the results back over to the theoreticians for the next round of predictions. By repeating these predictions and tests, the integrative methods are continually developed. The Research Center for Integrative and Computational Biology has continued to encourage and enhance interaction between theoretical biologists and experimental biologists.

The center provides equipment such as cluster machines and Unix-based machines for computation and experiments. Members can also use computational equipment at the Research Center for Computational Science in Okazaki. The center also provides experimental equipment for collaborations with experimental biologists.

Figure 2. A cluster machine

Over the last five years we have organized four meetings, including two international meetings on systems biology and mathematical biology that had many participants studying biological systems using different methods, including physics, mathematics and computational science. The meetings enhanced interactions between researchers in many different fields and resulted in several collaborative research projects.

CENTER FOR ANALYTICAL INSTRUMENTS (managed by NIBB)

Head
TAKADA, Shinji

Technical Staff:	*MORI, Tomoko* *MAKINO, Yumiko* *YAMAGUCHI, Katsushi* *TAKAMI, Shigemi* *OKA, Sanae* *TANIGUCHI, Misako*
Secretary:	*ICHIKAWA, Mariko*

The Center is responsible for amino acid sequence analysis, amino acid analysis, chemical syntheses of peptides, and next generation sequencing with SOLiD system (ABI) as well as supporting researchers at NIBB and NIPS. The Center's instruments can be used by researchers from outside the institute upon proposal.

Representative Instruments

Protein Sequencers (ABI Procise 494HT, 492cLC)
Amino Acid Analyzer (Hitachi L8500A)
Peptide Synthesizer (ABI 433A)
Plasmid Isolation Systems (Kurabo PI-50α, PI-50, PI-200)
Automatic Nucleic Acid Isolation System (Kurabo NA-2000)
Isoelectric Focusing System (Amarsham Biotech IPGphor)
Genetic Analyzers (ABI PRISM 3130xl, 310)
Real Time PCR System (ABI 7500)
Thermal Cyclers (ABI 9700, Perkin Elmer PJ-9600, Biometra TGRADIENT)
Particle Delivery System (Bio-Rad BiolisticPDS-1000/He)
Gas Chromatograph (Shimadzu GC-14APF-SC)
High Performance Liquid Chromatographs (Shimadzu LC-10AD, LC-6AD, Waters 600E, Alliance UV system)
Integrated Micropurification System (Pharmacia SMART)
Flow Cytometer (Coulter EPICS XL)
Biomolecular Interaction Analysis Systems (Pharmacia BIACORE 2000, Affinity Sensors IAsys)
Laboratory Automation System (Beckman Coulter Biomek 2000)
ESR Spectrometer (Bruker ER-200D)
GC/Mass Spectrometer (JEOL DX-300)
MALDI-TOF-MS (Bruker Daltonics REFLEX III)
LC/Q-TOF MS (Waters Q-TOF Premier)
Inductively Coupled Plasma Atomic Emission Spectrometer (Seiko SPS1200A)
Spectrofluorometers (Hitachi 850, F-4500, Simadzu RF-5000)
Spectrophotometers (Hitachi 330, 557, U-2001, Varian Cary5G, Perkin Elmer Lambda-Bio, NanoDrop ND-1000)
Microplate Luminometer (Berthold MicroLumat LB 96P)
Microplate Readers (Corona MTP-120, MTP-100F)
FT-IR Spectrophotometer (Horiba FT-730)
Bio Imaging Analyzer (Fujifilm BAS 2000)
Luminescent Image Analyzers (Fujifilm LAS 3000 mini)
Fluorescence Bio Imaging Analyzer (Takara FMBIO II)
Environmental Scanning Electron Microscope (PHILIPS XL30 ESEM)
Confocal Laser Scanning Microscopes (Leica TCS SP2, OLYMPUS FV1000)
Fluorescence Microscope (KEYENCE BZ-8000)
Color Laser 3D Profile Microscope (KEYENCE VK-8500)
High-Resolution Quick Microscope (KEYENCE VH-5000)

Figure 1. Protein sequencers

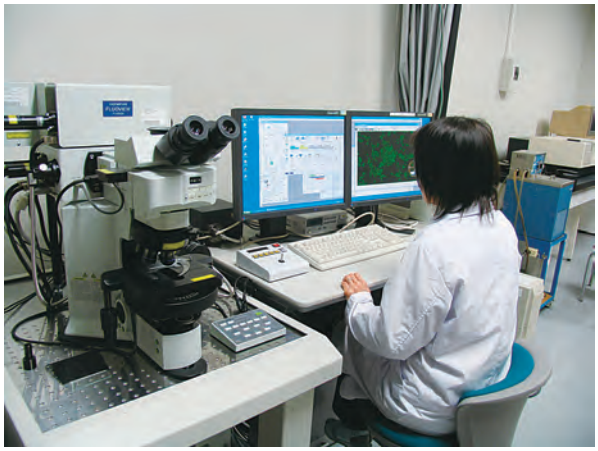


Figure 2. Confocal Laser Scanning Microscope

Figure 3. LC/Q-TOF-MS

CENTER FOR RADIOISOTOPE FACILITIES (OKAZAKI RESEARCH FACILITIES)

Head
HASEBE, Mitsuyasu

Associate Professor:	*OGAWA, Kazuo (Radiation Protection Supervisor)*
Technical Staff:	*MATSUDA, Yoshimi (Radiation Protection Supervisor)*
	SAWADA, Kaoru
	IINUMA, Hideko
Technical Assistants:	*ITO, Takayo*
	KAMIYA, Kiyomi

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

Matsuda, Iinuma, Ito, and Kamiya maintained CFBI (Common Facilities Building I)-branch and LGER (Laboratory of Gene Expression and Regulation)-branch in the Myodaiji-Area. Ogawa and Sawada worked in the Yamate-Area.

The number of registrants and the number of users from January 2009 to December 2009 are presented in Table 1.

Users counted by the monitoring system going in and out of the controlled areas numbered 3,764 persons during this period. The percentages and numbers for each area are shown in Figure 1 and Table 2. The annual changes of registrants and the number of totals per year are shown in Figure 2.

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

Thirty years have passed since the CFBI-branch opened and some parts of the air supply and exhaust system have deteriorated. Figure 3 shows the repair work done on the CFBI-branch to accommodate the law.

	Myodaiji-Area	Yamate-Area
registrants	134	88
users	69	39

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2009

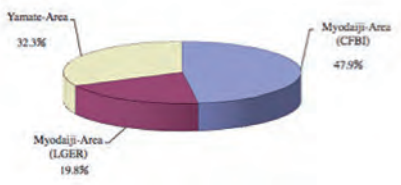


Figure 1. Percentage of users going in and out of each controlled area in 2009

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch		
users	1596	686	1094	3376
visitors	205	61	122	388
total	1801	747	1216	3764

Table 2. Users and visitors who entered each controlled area in 2009

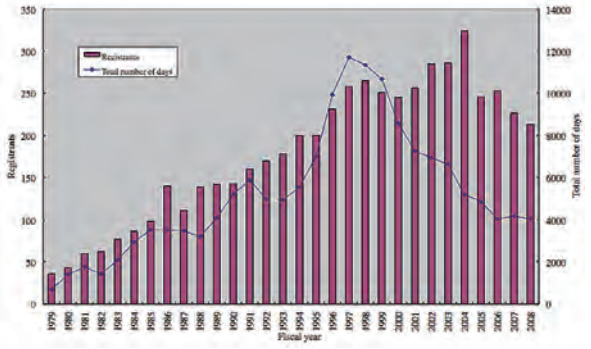


Figure 2. Annual changes of registrants and number of totals per fiscal year

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch		
^{125}I Receive	0	0	1205	1205
^{125}I Used	0	0	1114	1114
^{45}Ca Receive	0	0	-	0
^{45}Ca Used	0	0	-	0
^{35}S Receive	259000	0	20000	279000
^{35}S Used	66630	0	4000	70630
^{32}P Receive	1239000	176000	237000	1652000
^{32}P Used	992435	228600	192100	1413135
^{14}C Receive	66600	0	185	66785
^{14}C Used	27783	0	42	27825
^{3}H Receive	412550	0	6484250	6896800
^{3}H Used	53212	0	6578775	6631987

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2009

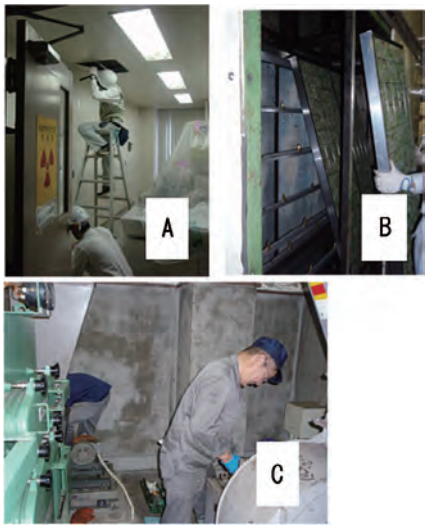


Figure 3. Repair work on air supply & exhaust system at CFBI-branch
A: The room re-heaters were replaced.
B: The air supply system's eliminators were replaced.
C: The exhaust fan was repaired.

STRATEGIC PLANNING DEPARTMENT

Chair
NISHIMURA, Mikio

Associate Professor: *KODAMA, Ryuji*

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

The main activities of the Department

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

2) Editing of the Annual Report (in collaboration with the Office of Public Relations and International Cooperation)
The department edits the annual report (this issue) which summarizes the annual activities of laboratories and facilities and is used in the evaluation procedures of the institute.

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

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

OFFICE OF PUBLIC RELATIONS AND INTERNATIONAL COOPERATION

Chair
UENO, Naoto

Assistant Professor	
(Specially appointed) :	*KURATA, Tomoko*
Technical Assistants:	*OTA, Misaki*
	TANAKA, Megumi
	MIYATA, Hiroe
	ADACHI, Shoko
	OTA, Kyoko
	EMDE, Jason R.
	KAWAGUCHI, Colin

The Office of Public Relations and International Cooperation, in order to communicate the activities of NIBB to the widest audience, performs both standard public relations duties as well as communication with scientific publications and organizations worldwide. The Office of Public Relations and International Cooperation also manages the planning and administration of the international symposiums and international training courses held at NIBB.

The main activities of the office in 2009

1) Press releases

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

2) Supporting international conferences

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

3) Management of education related programs

The Fourth International Practical Course (June, 2009)
Bioinformatics training course (August and September, 2009)

4) Updating and maintenance of the NIBB web page

5) Editing of publications, production of posters and leaflets

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

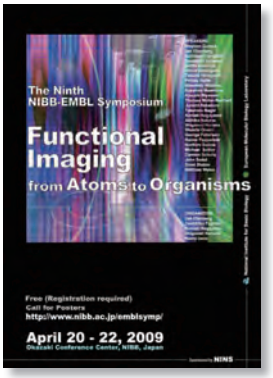
6) Organization of scientific outreach programs

Management of collaborative events with Japan's National Museum of Emerging Science and Innovation. Assisting exhibitions held at the Nagoya City Scientific Museum.

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

Name	*Date*	*Title*	*Organizer*
9th NIBB-EMBL joint symposium	April 20-22	Functional Imaging from Atoms to Organisms	J. Ellenberg, K. Nagayama, N. Ueno
1st NIBB-MPIZ joint symposium	August 25-27	Japanese-German Symposium on Evolution and Development	M. Koornneef and K. Okada

Table 1. International conferences managed by the Office of Public Relations and International Cooperation in 2009



Ninth NIBB-EMBL Symposium

Open Campus 2009

Graduate School Showcase 2009

Passionate about Science

Figure 1. Examples of posters, pamphlets and abstract books produced by the Office of Public Relations and International Cooperation in 2009

TECHNICAL DIVISION

Head
FURUKAWA, Kazuhiko

Common Facility Group

Chief: *MIWA, Tomoki*

● ***Research Support Facilities***

Unit Chief: *HIGASHI, Sho-ichi*
Subunit Chief: *NANBA, Chieko*
Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
Technical Assistants: *SUZUKI, Keiko*
ICHIKAWA, Chiaki
TAKESHITA, Miyako
NISHIMURA, Noriko
YAMAMOTO, Kumi
ENDOU, Seiichiro
Secretary: *ISHIKAWA, Azusa*

● ***Center for Analytical Instruments***

Unit Chief: *MORI, Tomoko*
Subunit Chief: *MAKINO, Yumiko*
YAMAGUCHI, Katsushi
Technical Staff: *TAKAMI, Shigemi*
TANIGUCHI, Misako
Secretary: *ICHIKAWA, Mariko*

● ***Transgenic Animal Facility***

Subunit Chief: *HAYASHI, Kohji*
Technical Staff: *NOGUCHI, Yuji*
Technical Assistants: *ICHIKAWA, Yoko*
TAKAGI, Yukari

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

Unit Chief: *MATSUDA, Yoshimi*

● ***Radioisotope Facility***

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

Research Support Group

Chief: *KAJIURA-KOBAYASHI, Hiroko*

● ***Cell Biology***

Unit Chief: *KONDO, Maki*

● ***Developmental Biology***

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

● ***Neurobiology***

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

● ***Evolutionary Biology and Biodiversity***

Unit Chief: *FUKADA-TANAKA, Sachiko*
Subunit Chief: *KABEYA, Yukiko*
Technical Staff: *MOROOKA, Naoki*

● ***Environmental Biology***

Unit Chief: *MIZUTANI, Takeshi*

Reception

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

The Technical Division is a support organization for researchers and research organizations within the 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 the 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.

NIBB-EMBL Meetings

The 9th NIBB-EMBL Joint Meeting: "Functional Imaging from Atoms to Organisms"

April 20 (Mon)-22 (Wed), 2009 | Okazaki Conference Center, Okazaki

This joint meeting was the second bioimaging-related meeting of the NIBB-EMBL joint meeting series following the first symposium "Frontiers of bioimaging" held in 2006, and aimed to overview the recent advance of bioimaging from the molecular to the organismal level. In contrast to the previous meeting in which light microscopy technologies focusing on cell functions and animal and plant development were discussed, in this meeting many cutting edge technologies of single molecule imaging at high resolutions with electron as well as light microscopy combined with quantitative analyses to capture molecular and cellular dynamics were presented. The talks illustrated the rapid advance of bioimaging during the last three years and outlined the future direction of bioimaging using new methodologies of electron and light microscopy, imaging-based large scale screening, and image processing for quantitative analysis of a diverse range of biological phenomena. Several short oral presentations were selected from posters and four young scholars including graduate students of EMBL participated in the meeting as a part of the student exchange program of the NIBB-EMBL collaboration, both of which further stimulated the meeting and contributed to the success of the symposium.

Speakers

Cusack, Stephen (EMBL Grenoble), Ellenberg, Jan (EMBL Heidelberg), Frangakis, Achilleas (Univ. Frankfurt), Keller, Philipp (EMBL Heidelberg), Mueller-Reichert, Thomas (Max Planck Inst.), Patterson, George (NIH), Sattler, Michael (Helmholtz Zentrum Munchen & TU Munchen), Schultz, Carsten (EMBL Heidelberg), Sedat, John (UCSF), Stelzer, Ernst (EMBL Heidelberg), Weiss, Matthias (DKFZ) Fujimori, Toshihiko (NIBB), Goshima, Gohta (Nagoya Univ.), Hamaguchi, Hiro-o (Univ. Tokyo), Haraguchi, Tokuko (KARC), Kikkawa, Masahide (Kyoto Univ.), Maeshima, Kazuhiro (NIG), Miyawaki, Atsushi (RIKEN / JST), Nabekura, Junichi (NIPS), Nagai, Takeharu (Hokkaido Univ.), Nagayama, Kuniaki (OIB), Nakano, Akihiko (Univ. Tokyo), Nonaka, Shigenori (NIBB), Onami, Shuichi (RIKEN ASI), Sadato, Norihiro (NIPS), Ueno, Naoto (NIBB)

Invited Students from EMBL
Blattmann, Peter; Cobos Correa, Amanda; Heiligenstein, Xavier; Wuensche, Annelie

Comments from the Students

Xavier Heiligenstein (EMBL)

I found the content of the conference really interesting and of a great level. The comments, questions and discussion to me really facilitated going deeper in to each presentation. I got a great and deep overview of what light microscopy can offer us today in complement to electron microscopy and also enjoyed the external activities a lot, such as visiting the NIBB institute, its spectrophotometer and its brand new Light Sheet Microscope. Having the opportunity to give a short talk was also nice and lead to good feed-back during the poster session. Complementing this nice meeting, we had some great interaction with the Post-doc and Students and also greatly enjoyed the side parts of this symposium (a very nice party on Tuesday evening). And what to say about the excursion that lead us to see wonderful landscapes, cultural, and scientific Japanese pride. Again I would like to thank you again for such a nice welcome.

Peter Blattmann (EMBL)

I enjoyed the 9th NIBB-EMBL Symposium on Functional Imaging a lot. For me, it was an invaluable experience to come to Japan and attend this conference. I was amazed by the high standards of all the talks and discussions throughout the conference and profited a lot from them. The program was very well chosen and I think I received a good and interesting overview of the cutting edge research going on in this field. The hospitality with which we were received was outstanding and I felt very welcome at all times. However, for me personally, the greatest experience was to meet the Japanese students, post-docs and the speakers and thus to learn about the Science and technology conducted in Japan. I am very grateful to have received the opportunity to present both my poster and also a short talk at this conference. The following excursion was also very enjoyable and for the first time I experienced Japanese culture and mountains, which I liked very much. I am very glad, that this fruitful collaboration between NIBB and EMBL is continuing and look forward to further opportunities to interact with NIBB members. Thank you again so much for the hospitality and all your efforts!

NIBB-EMBL Meetings

The 1st NIBB-EMBL PhD Mini-Symposium and 11th International EMBL PhD Students Symposium

October 28 (Wed)-31 (Sat), 2009 | EMBL, Heidelberg

The first NIBB-EMBL PhD Mini-Symposium was held at EMBL, Heidelberg on 28 October. Seven PhD students from NIBB and three from Nagoya University got together with EMBL pre-docs to exchange and discuss their recent results. This symposium and subsequent "lab tour" also connected our students with EMBL PIs who are in the field of their respective interests. On 29-31 October the 11th International EMBL PhD Students Symposium "Puzzles in Biology; Putting the Pieces Together" was held at EMBL. The program contained three sessions; the first aimed to shed light on the latest technical innovations in the field of biology, the second session focused on complex puzzles which had been resolved by integrating data from different scientific fields, and the final session covered several issues which illustrated beneficial relationships between basic biology and applied studies to understand the mechanisms causing human diseases. Our PhD students presented their research work during the poster and the short-talk session. It was a great opportunity for our PhD students to meet students from a variety of European countries and hear about their interesting work. Moreover, they were very much impressed by the scientific atmosphere and cutting-edge researches at EMBL. We hope to continue this collaboration in the future.

Participating students from Japan

Hara, Yusuke; Morita, Hitoshi; Okamoto, Haruko; Goto, Shino; Tameshige, Toshiaki; Sugimoto, Ryo; Takahashi, Hiroyuki (NIBB); Yamamoto, Haruki; Mollah, Md.Bazlur Rahman; Tanimoto, Masashi (Nagoya Univ.)

Comments from students (excerpts)

・Takahashi, Hiroyuki

This was my first experience of an English oral presentation in a foreign country and its preparation was a tough job for me. This opportunity of an oral presentation was, however, a precious one and gave me experience in discussing my research with EMBL people. A student of EMBL was assigned as a guide for each student from Japan, which made it easier to communicate with EMBL students and to have a glimpse of the students' life at EMBL and their view of research. I found students at EMBL are much more aggressive and positive in their research than I had expected, which I think we should follow.

・Tameshige, Toshiaki

At the 11th International EMBL PhD Student Symposium, there were a lot of interesting presentations by researchers from various fields, but the most surprising thing was that the whole symposium was organized by the EMBL students. From the invited speakers, I can easily imagine how widely the EMBL students' knowledge and interest range. And I thought it was a fruitful experience for them to organize such a big symposium while still learning as students.

・Haruko, Okamoto

The best thing I obtained at this Ph.D symposium was that I had an opportunity to communicate with students of similar ages from various countries. I spoke with students from countries unfamiliar to me, such as Israel and Nigeria. I experienced the everyday life of students in Europe, such as dance parties and talks over drinks. I found, contrary to my expectation, we have much in common.

The 1st NIBB-MPIZ Joint Symposium Japanese-German Symposium on Evolution and Development

Max Planck Institute for Plant Breeding Research, Cologne, Germany

August 25(Tue)-27(Thu), 2009

NIBB formalized an Agreement with Max-Planck Institute for Plant Breeding Research (MPIZ, at Cologne, Germany) in April, 2009. This agreement aims to promote intellectual, educational and technological exchange between researchers and students of Japan and Germany by joining NIBB and MIPZ as national hubs of scientific cooperation in the field of plant science. As the first joint activity, a Japanese-German Symposium on Evolution and Development was held at MPIZ in Cologne from August 25 to 26.

NIBB began gathering applicants who wished to attend the symposium and are planning possible collaborative research with researchers of MPIZ through e-mail networks, and selected seven researchers from the applicants. At the symposium the Japanese delegation, consisting of the seven applicants and five PIs of NIBB plant science laboratories, participated in discussions with researchers and students of MPIZ, Cologne University, and other universities and institutes of Germany. After the symposium, on the morning of August 27, individual talks were held between Japanese and German researchers with the aim of starting possible collaborative research. In the afternoon of August 27, Japanese researchers visited the Demonstration Garden and Wissenschaftsscheune (Science Barn) of MIPZ, which was founded for the purpose of giving the public an opportunity to experience several aspects of plant science including Mendel's law, domestication of crops, and GMO plants.

As a continuing activity, NIBB supports the traveling costs of three Japanese researchers who visit MIPZ for collaborative research projects which arose from attending the Symposium.

The NIBB International Practical Course

The first NIBB International Practical Course was held in 2007 with the aim of providing young scientists around the world with opportunities to learn cutting-edge experimental skills and to communicate with experts in the field. The course replaced our Bioscience Training Course, which had been held for twenty years and which had, for the most part, accepted participants from universities and institutes in Japan. The Fourth International Practical Courses was held in 2009 as summarized below. The course was held in a laboratory provided by NIBB and equipped with the necessary instruments and tools and a good supply of experimental materials. We hope that the techniques and knowledge provided in these courses will prove useful in the future work of all of the participants.

The Fourth International Practical Course: " 2009 NIBB Laboratory Course and Workshops on *Physcomitrella patens*"

- Period: June 29 (Mon)- July 3 (Fri), 2009
- Participants: 17 (five from Japan, two from the UK, Germany, and Israel, and one each from Belgium, Czech, France, Sweden, Singapore, and India)
- Lectures:
  Dr. Andrew C. Cuming (Univ. Leeds)
  Dr. Yasuko Kamisugi (Univ. Leeds)
  Dr. Tomoaki Nishiyama (Kanazawa Univ.)
  Dr. Tetsuya Kurata (JST)
  Dr. Yoshikatsu Sato (JST)
  Dr. Yuji Hiwatashi (NIBB)
  Dr. Minoru Kubo (JST)
  Dr. Takashi Murata (NIBB)
  Dr. Mitsuyasu Hasebe (NIBB)

Contents of the course: basic techniques including cultivation, observation of developmental processes at the cellular level, gene targeting, bioimaging, and bioinformatics used in the biology of the moss *Physcomitrella patens*

The NIBB Training Course "How to translate results from microarray analysis"

NIBB started a series of training courses on up-to-date research techniques for researchers from mainly Japanese universities and institutions. In 2009 we held a bioinformatics training course on the statistical analyses of data obtained by microarray analyses.

- Period: August 19 (Wed) -21 (Fri), 2009
  September 8 (Tue) -10 (Thu), 2009
- Lecturers: Drs. Koji Kadota and Yuji Nakai (University of Tokyo)
- Participants:
  August course: 16 (including 5 from NIBB)
  September course: 17 (including 4 from NIBB)

Microarray analysis is one of the most frequently used comprehensive methods for researching gene expression. But it is not easy to properly translate results from microarray data for ordinary biologists who are not familiar with bioinformatics. The aim of this course was to give lectures on the fundamentals of microarray analysis using the statistical environment "R", and practical methods for gene ontology analysis using free software.

1. Fundamentals of microarray analysis
2. How to use the statistical environment "R" for microarray analysis
3. Fundamentals of gene ontology analysis
4. Practical methods for gene ontology analysis

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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

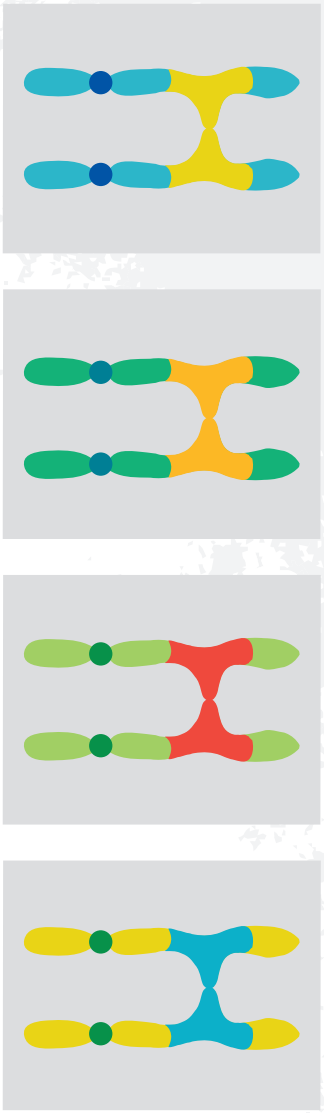
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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