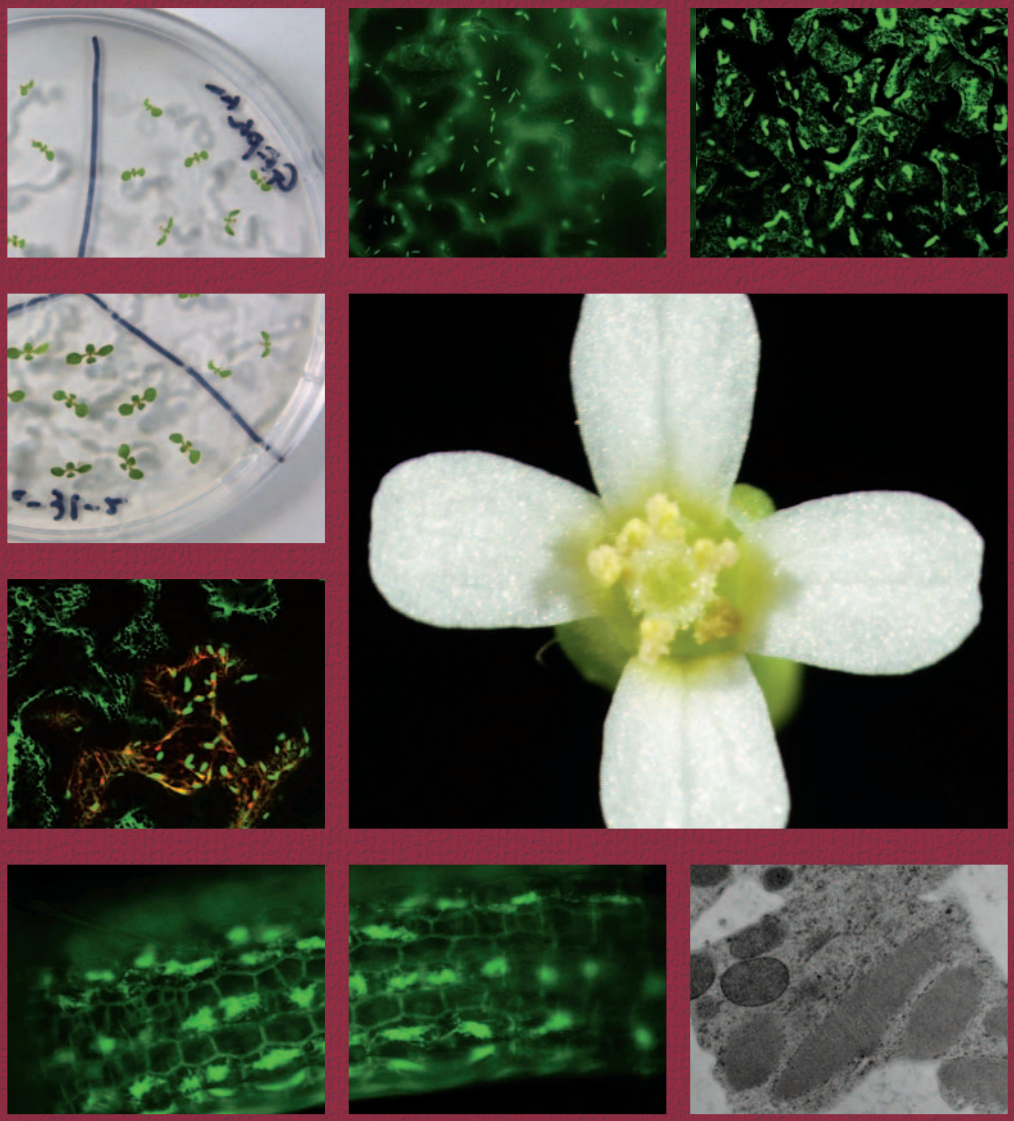


National Institute for Basic Biology

2008 ANNUAL REPORT

CONTENTS

The cover photographs include fluorescent images of ER-targeted GFP in *Arabidopsis thaliana* seedlings. Endoplasmic reticulum (ER) body, a novel plant organelle with a rod-shaped structure in epidermal cells, accumulates β-glucosidase and is involved in a defense system against pathogens. NAI2 is identified as an ER body component for the ER body formation and the accumulation of β-glucosidase, See The Plant Cell (2008) volume 20, page 2529-2540 and page 9 of this report for details.

Kiyotaka Okada

Our institute, the National Institute for Basic Biology (NIBB), has developed as a center of excellence in research, education, and inter-university collaboration in the various fields of basic biology since its foundation in 1977. In 2004, NIBB and four national research institutes established a new organization, the National Institutes of Natural Sciences (NINS), one of the four Inter-University Research Institute Corporations. In 2008, we gladly had two professors joining our institute, and are now covering a wide variety of fields including cell biology, developmental biology, neurobiology, evolutionary biology and biodiversity, environmental biology, theoretical biology and imaging science, and are studying the basic action of genes and cells common to all living organisms, as well as the mechanisms involved in producing the diversified structures and activities of life adapting to an ever-changing environment. Research in these basic biological fields is maintained at a high level while we continue to search for new and challenging fields of study. We are also involved in ongoing collaboration with a number of researchers from Japanese and foreign universities and institutes. In addition to research, the education of the next generation of researchers is another important aim. As a member of the Graduate University for Advanced Studies (SOKENDAI), we are involved in training graduate students. We will have three new doctors in March of 2009.

NIBB's activities during 2008, as well as our goals for the future, are summarized in this booklet. We are always open to questions, comments and suggestions.

Kiyotaka OKADA, D. Sci.
Director-General, NIBB
Jan 12, 2009

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, 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 Director-Generals, faculty appointments, NIBB's annual budget and future prospects.

The Strategic Planning Department is a central office for public relations and the management of conferences and other extramural activities. The department also assists the Director-General with NIBB's evaluation procedures and in planning long-range strategies for the institute.

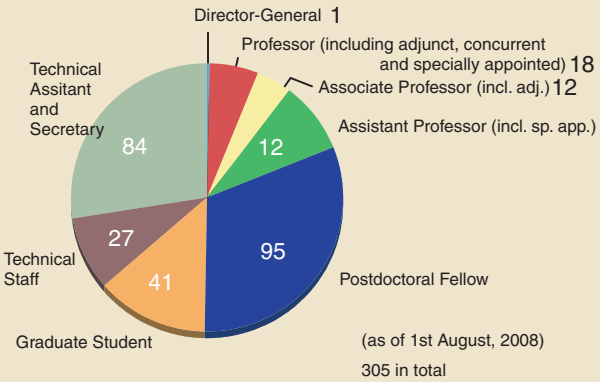
Administration of NIBB is undertaken by the Okazaki Administration Office under the direct auspices of the Administration Office of NINS.

Research and Its Support

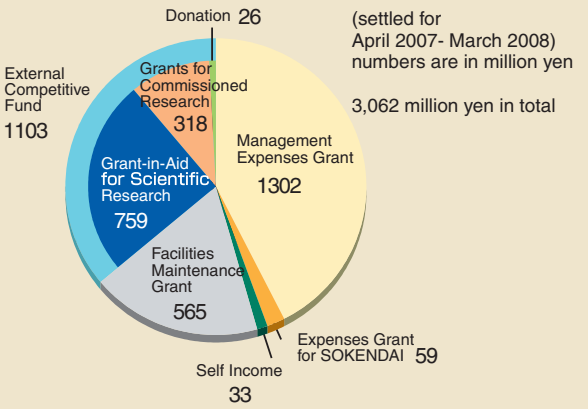
NIBB conducts its research programs through thirty-one research units and three research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. Some 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 throughout Japan. The Technical Division manages the activities of the technical staff and helps to promote the research activities of each research unit and to maintain the common research resources of both the research support units of NIBB and some of 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 function also as NIBB's divisions.

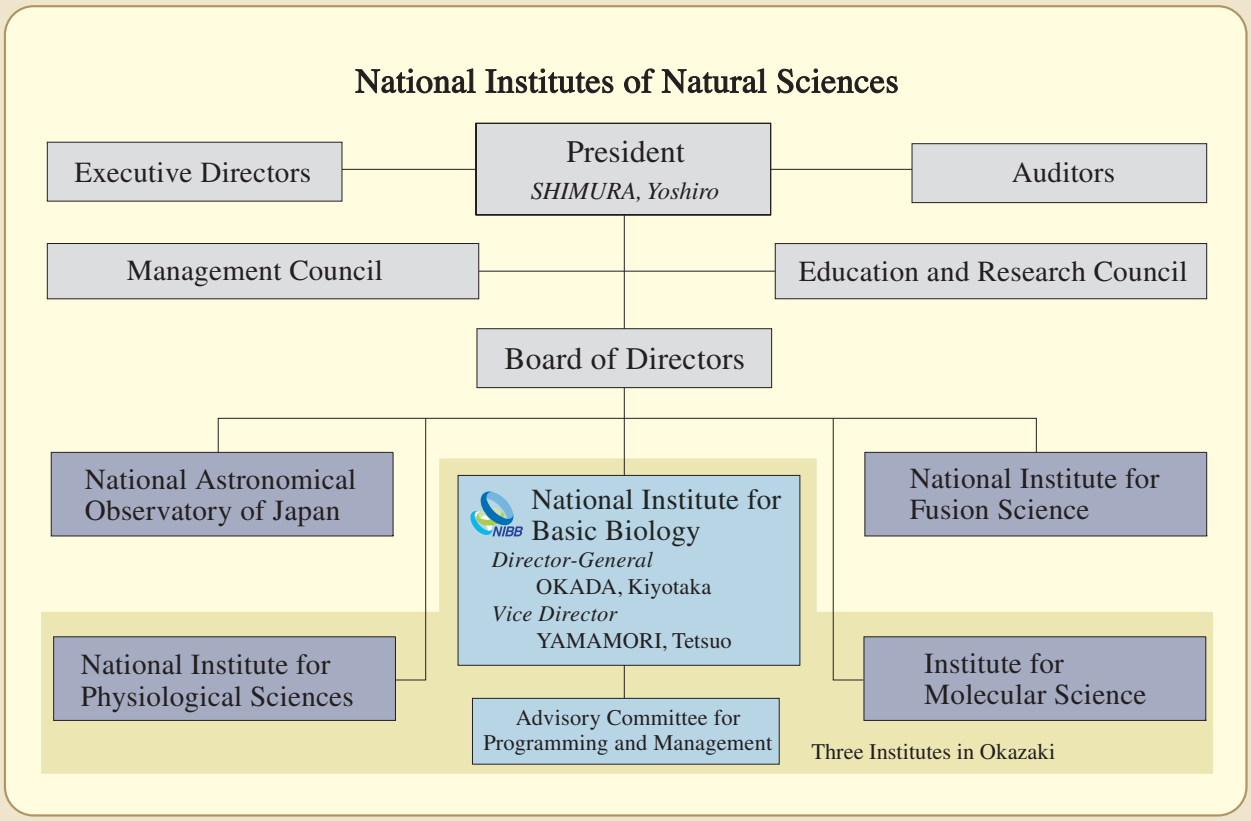
Personnel in NIBB



Financial Configuration of NIBB



Organization



Members of the Advisory Committee for Programming and Management (as of April 1st, 2008)

Chairperson	OHSUMI, Yoshinori	Professor, National Institute for Basic Biology
Vice-Chair	IWASA, Yoh	Professor, Kyushu University
Non-NIBB member	HASEGAWA, Mariko	Professor, The Graduate University for Advanced Studies (SOKENDAI)
	ISHINO, Fumitoshi	Professor, Tokyo Medical and Dental University
	KATO, Kazuto	Associate Professor, Kyoto University
	MATSUOKA, Makoto	Professor, Nagoya University
	OSUMI, Noriko	Professor, Tohoku University
	SAKANO, Hitoshi	Professor, The University of Tokyo
	SATOH, Noriyuki	Professor, Kyoto University
	TABATA, Satoshi	Deputy Director, Kazusa DNA Research Institute
NIBB member	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	HORIUCHI, Takashi	Professor, National Institute for Basic Biology
	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
	IIDA, Shigeru	Professor, National Institute for Basic Biology
	KOBAYASHI, Satoru	Professor, Okazaki Institute for Integrative Bioscience
	NISHIMURA, Mikio	Professor, National Institute for Basic Biology
	NODA, Masaharu	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, Okazaki Institute for Integrative Bioscience
	UENO, Naoto	Professor, National Institute for Basic Biology
	YAMAMORI, Tetsuo	Professor, National Institute for Basic Biology

National Institute for Basic Biology

Research Units

- **Cell Biology**
  - Division of Cell Mechanisms
  - Division of Molecular Cell Biology
  - Division of Cell Proliferation (adjunct)
  - Laboratory of Cell Structure
  - Laboratory of Cell Sociology
- **Developmental Biology**
  - Division of Morphogenesis
  - Division of Developmental Genetics †
  - Division of Molecular and Developmental Biology ††
  - Division of Embryology
  - Division of Germ Cell Biology
  - Division of Reproductive Biology
  - Division for Sex Differentiation
  - 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 Molecular Genetics
  - Division of Genome Dynamics
  - Division of Evolutionary Biology
  - Laboratory of Morphodiversity
  - Laboratory of Bioresources
- **Environmental Biology**
  - Division of Molecular Environmental Endocrinology †††
  - Division of Plant Developmental Genetics (adjunct)
  - Division of Photobiology (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**

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 ***
  - Department of Strategic Methodology
- **Center for Radioisotope Facilities**
- **Center for Experimental Animal**
- **Research Center for Computational Science**

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

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 envision contributing to the world-wide community of biologists through our efforts to accomplish these goals. This chapter briefly explains four of the 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 to be 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 will facilitate the strategic organization of collaborative research projects. "Priority collaborative research projects" are carried out in one to three years as group research by internal and external researchers with the purpose of developing pioneering research fields in biology. Six projects have already been carried out, including "molecular mechanisms for controlling the individuals of higher plants." The category "collaborative research projects for model organism/technology development" was established in 2007 with the aim of developing and establishing new model organisms. Five projects have already been carried out, including "development of the transgenic strain of *Cabombaceae* (primitive angiosperm)." NIBB always encourages discussion on such projects in the belief that the 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	2005	2006	2007	2008
Priority collaborative research projects	2	3	1	0
Collaborative research projects for model organisms / technology development	–	–	2	3
Individual collaborative research projects	41	37	43	49
NIBB workshops	4	1	5	5
Collaborative experiments using the Large Spectrograph	19	18	14	11
Facility Use (Center for Analitical Instruments)	0	0	1	0
total	66	59	66	68

Enhancement of the Large Spectrograph

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

International Cooperation

Collaborative research projects 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 81 for details of the NIBB-EMBL joint meetings held in 2008).



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

NIBB Conference

The NIBB Conference is an international conference organized by NIBB's professors once or twice a year with the participation of a guest lecturer 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 (see page 78 for details of the NIBB Conferences held in 2008).

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 second and third courses were held in 2008 (see page 83). Graduate students and young researchers from various areas including China, Hong Kong, Taiwan, and Korea, 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.

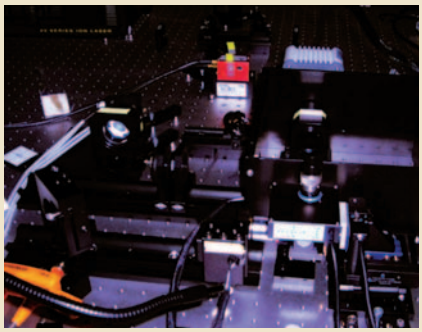
Top: Medaka strain for which the full genome sequence was determined,
Middle: Transgenic Medaka with red fluorescence,
Bottom: A strain with transparent body.

Development of New Fields of Biology

Bioimaging

Recently, the capability of optical microscopes has greatly improved, and biophotonic probes have also 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) in biological research and to develop new imaging techniques.

1) *Imaging Science Laboratories*, NIBB aims to be a center for developing 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 DSLM* (*Digital Scanned laser Light sheet Microscopy*), As part of collaborative work with EMBL, NIBB has introduced 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 exchanges 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

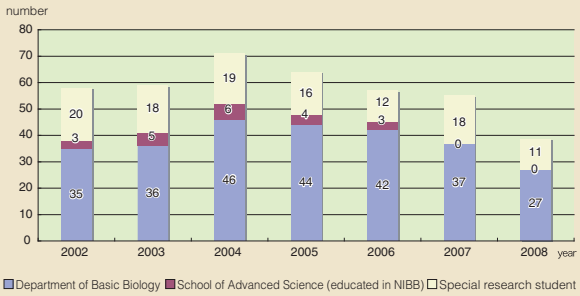
   NIBB constitutes the Department of Basic Biology in the School of Life Science of the Graduate University for Advanced Studies (SOKENDAI). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree.

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.

Graduate students educated by NIBB



Personnel changes in 2008*

Newly assigned in NIBB

Name	Position	Research Unit	Date
TATEMATSU, Kiyoshi	Assistant Professor	Laboratory of Plant Organ Development	March 1
HAYASHI, Yoshiki	Assistant Professor	Division of Developmental Genetics	April 1
KURATA, Tomoko	Assistant Professor (Specially appointed)	Strategic Planning Department	April 1
FUJIMORI, Toshihiko	Professor	Division of Embryology	August 1
YOSHIDA, Shosei	Professor	Division of Germ Cell Biology	August 1
TOYOOKA, Yayoi	Assistant Professor	Division of Embryology	October 1
SUZUKI, Makoto	Assistant Professor	Division of Morphogenesis	October 1

Newly affiliated in other universitites and institutes

Name	New Affiliation	Position	Date
IMAMURA, Takuya	Kyoto University	Associate Professor	February 29
OKUBO, Kataaki	University of Tokyo	Project Assistant Professor	March 31
TANAHASHI, Takako	Japan Patent Office	Jr. Patent Examination Official	March 31
NAKAMURA, Makoto	Matsuyama University	Associate Professor	March 31
MOCHIZUKI, Atsushi	RIKEN Advanced Research Institute	Chief Scientist	June 30
OGAWA, Hidesato	Natl. Inst. Inform. Commun. Tech.	Research Scientist	July 31

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

Awardees in 2008

Name	Position	Award
KITADATE, Yu	Postdoctoral Fellow	Inoue Research Award for Young Scientists
SHIMIZU, Hidetada	Postdoctoral Fellow	SOKENDAI's Nagakura Research Incentive Award
YAMAGUCHI, Takahiro	Assistant Professor	The Young Scientist Award for Plant Morphology
HASEBE, Mitsuyasu	Professor	The Research Award of the Botanical Society of Japan
FUJIKAWA, Akihiro et al.	Postdoctoral Fellow	The JB Award of the Japanese Biochemical Society
EGUCHI, Goro	Professor Emeritus	The Order of the Sacred Treasure, Gold and Silver Star

Note: On the unit member lists from P. 8 to P. 77 all members who belonged to the unit during 2008 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 changed during 2008. 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
Postdoctoral Fellows	ARAI, Yuko
	KAMIGAKI, Akane
	OIKAWA, Kazusato
	SINGH, Tanuja
Graduate Students	OGASAWARA, Kimi
	KANAI, Masatake
	GOTO, Shino
	CUI, Songkui
	SHIBATA, Michitaro
Technical Assistants	NAKAMORI, Chihiro
	YOSHINORI, Yumi
	SUZUKI, Iku
	FUKAZAWA, Mitsue
	KATO, Kyoko
	NISHINA, Momoko
	SATO, Yori
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" was started to clarify the molecular mechanisms underlying organelle differentiation.

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 *Arabidopsis* genome. Custom-made DNA microarray covering all these genes was used to investigate expression profiles of the peroxisomal genes in various organs. It revealed that peroxisomes in root cells plays a pivotal role in polyamine catabolism. We also made a two-dimensional protein map of glyoxysomes and leaf peroxisomes isolated from *Arabidopsis* and soybean. Peptide MS fingerprinting analyses allowed us to identify novel peroxisomal membrane proteins, i.e. voltage-dependent anion-selective channel and adenine nucleotide carrier 1 (PNC1) (Figure 1). The overall results provide us new insights into plant peroxisomal functions.

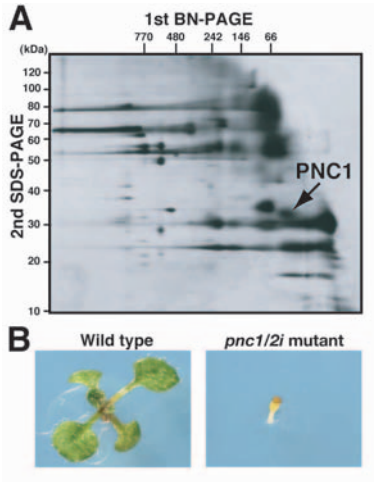


Figure 1. Proteomic identification and characterization of a novel peroxisomal adenine nucleotide carrier 1 (PNC1). (A) 2-D map of proteins in the digitonin-soluble fraction of purified peroxisomes. A 2-D gel in which the first dimension was Blue Native (BN)-PAGE and the second was SDS-PAGE is shown. Protein spots were detected by silver staining. (B) Phenotype of the *pnc1/2* RNAi knockdown mutant. Seedlings grown on medium without sucrose under constant illumination for 7 days.

Bioinfomatic analysis of *Arabidopsis* genome predicted the presence of 15 kinds of genes, called *PEX* genes, for peroxisomal biogenesis factors. We 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. Of these, PEX5 and PEX7 form a cytosolic receptor complex and recognize PTS1- and PTS2-containing proteins, respectively. PEX14 is a peroxisomal membrane docking protein that captures the receptor-cargo complex. We continue to investigate the detailed molecular functions of other *PEX* genes.

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 than 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. Two other *apm* mutants, *apm2* and *apm4*, showed GFP fluorescence in the cytosol as well as in peroxisomes. We demonstrated that both mutants are defective in PTS1- and PTS2-dependent protein transport to peroxisomes, and that *APM2* and *APM4* encode proteins homologous to PEX13 and PEX12, respectively. It was revealed that APM2/PEX13 and APM4/PEX12 are components of the protein-translocation machinery on peroxisomal membranes, and that they are involved in protein transport from the cytosol into the peroxisome. Other *APM* mutants are currently under investigation in order 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 (5 μm long and 0.5 μm wide) 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* mutant has no ER bodies in whole plants and does 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 member of a unique protein family that is only found in the Brassicaceae. NAI2 localizes to the ER body. In the *nai2* mutant, PYK10 becomes uniformly diffused throughout the ER (Figure 2). These findings indicate that NAI2 is a key factor that enables ER body formation and the accumulation of PYK10 in ER bodies.

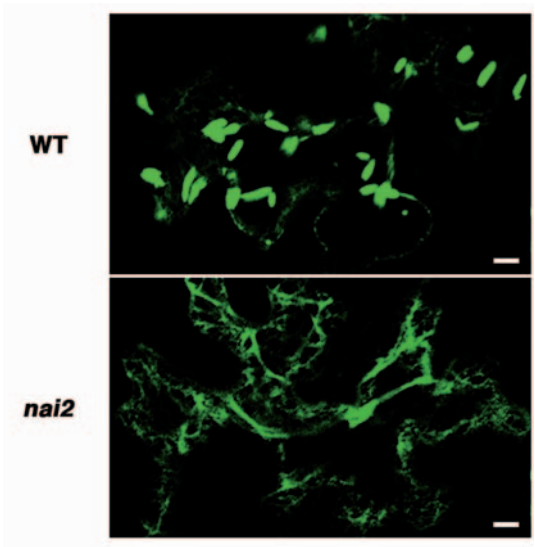


Figure 2. Localization of GFP-PYK10 in *nai2* mutant. GFP-PYK10 fusion gene was transiently expressed in the epidermal cells of 6-day-old wild-type (WT) and *nai2* seedlings. GFP-PYK10 is localized in ER bodies in wild-type plants; it becomes uniformly diffused, however, in ER in the *nai2* mutant. Bars = 10 μm.

Ⅴ. Vacuolar processing enzyme 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 programmed cell death (PCD) in plants. VPE is identified as the proteinase that exhibits caspase 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. Plants evolve a death strategy mediated by a vacuolar system, which is not seen in animals. Interestingly, a vacuolar enzyme is the key player in a 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 the genes with heat shock response element (HSE) motifs in their

promoters, suggesting that heat shock transcription factor (HSF) is involved in the response. *Arabidopsis* HSFs interacted with HSP90. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. Upon 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 (PODB) was built to promote a comprehensive understanding of organelle dynamics and is maintained by the Scientific Research of Priority Areas on "Organelle Differentiation as the Strategy for Environmental Adaptation in Plants." We released Version 2 (PODB2), which incorporated time-lapse and 3D images. PODB2 consists of 4 individual parts: the organelles movie database, the organellome database, the functional analysis database, and external links (Figure 3). The organelles movie database contains the videos for organelle movements and 3D structures. 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. This public database is open to all researchers. We expect that this database will be a useful tool to help researchers gain greater knowledge of plant organelles.

Figure 3. The graphical user interfaces of PODB2 (http://podb.nibb.ac.jp/Orgenellome).

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〔Review articles〕

- Arai, Y., Fukao, Y., Hayashi, M., and Nishimura, M. (2008). Peroxisome. In Plant Proteomics: Technologies, Strategies and Applications, Agrawal, G. K., and Rakwal, R. eds. (John Wiley & Sons, Inc.) pp. 377-389.
- Yamada, K., and Nishimura, M. (2008). Cytosolic heat shock protein 90 regulates heat shock transcription factor in *Arabidopsis thaliana*. Plant Signaling & Behavior *3*, 660-662.

DIVISION OF MOLECULAR CELL BIOLOGY

Professor
OHSUMI, Yoshinori

Assistant Professors	KAMADA, Yoshiaki
	SUZUKI, Kuninori
	NAKATOGAWA, Hitoshi
Technical Staff	KABEYA, Yukiko
NIBB Research Fellows	OBARA, Keisuke*
	HANADA, Takao
Postdoctoral Fellows	OBARA, Keisuke
	HANADA, Takao*
	FUJIKI, Yuki
	OKU, Masahide
	HARASHIMA, Toshiaki
	YAMAMOTO, Hayashi
	OKAMOTO, Koji
	KAKUTA, Soichiro
	KOBAYASHI, Takafumi
Graduate Students	KAGEYAMA, Takuya
	OH-OKA, Kyoko
Technical Assistants	KONDO, Chika
	NIIMI, Kaori
	ISHII, Junko
	OKAMOTO, Noriko
	ICHIKAWA, Rie
Secretaries	HARA, Yoko
	SUZUKI, Yuko

Most cellular activity is maintained by the balance between the synthesis and degradation of related proteins. Therefore, degradation processes as well as the regulation of gene expression play important roles in many physiological aspeets. 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. Lap3 is a selective target of autophagy in yeast

Although autophagy is, in principle, a non-selective degradation process, several proteins and organelles were found to be selectively incorporated into the autophagosome. For instance, Ald6, a soluble cytoplasmic enzyme in yeast, is preferentially eliminated from the cytoplasm via autophagy; Little is known, however, about the mechanisms of Ald6 targeting to autophagosomes. Autophagy is also involved in the selective transport of Ape1, a vacuolar hydrolase, as a biosynthetic route. Recently, we discovered that leucine aminopeptidase III (Lap3), a soluble cytosolic cysteine protease, was spatially associated with Ape1 and selectively transported to the vacuole during nitrogen starvation (Figure 1). The rate of Lap3 transport was much higher than that of Ald6 and was similar to that of Ape1. Moreover, Atg11 and Atg19, essential factors for Ape1 transport, were important for Lap3 transport. Most of the Lap3 was degraded within a couple of hours in the vacuole, in contrast to Ape1; therefore, we concluded that the machinery required for Ape1 biosynthesis is used for the selective degradation of Lap3.

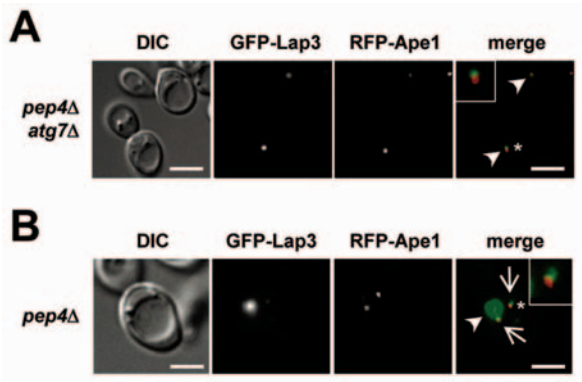

Figure 1. Microscopic analyses of Lap3. (A) Localization of GFP-Lap3 in *pep4Δ atg7Δ* (A) and *pep4Δ* (B) cells. Cells were grown in YPD media and then incubated in nitrogen starvation media (SD-N) for 3 h. The GFP and mRFP signals were observed simultaneously. Arrowheads indicate the co-localization of Lap3 and Ape1. Arrows point to intravacuolar structures. Insets are high-magnification images of dots marked with asterisks. GFP-Lap3 and mRFP-Ape1 dots do not merge, but are localized very closely to each other. Bars, 5 μm.

Ⅲ. Structural basis of cargo recognition by Atg8 homologs in selective autophagy

Atg8 homologs are ubiquitin-like proteins that localize on autophagosomal membranes and play crucial roles in the formation of the membranes. These proteins are also involved in the selective incorporation of specific cargo molecules into autophagosomes, in which Atg8 and its mammalian homolog, LC3, interact with Atg19 and p62, receptor proteins for a number of vacuolar enzymes including Ape1 and disease-related, ubiquitin-positive protein inclusions, respectively. In collaboration with Dr. Inagaki's group at Hokkaido University, we determined the structures of Atg8 and LC3 in complex with Atg19 and p62 peptides, respectively, which contain amino acid residues necessary and sufficient for complex formation. Remarkably, Atg8 and LC3 proved to interact with Atg19 and p62 in a quite similar manner, even though the entire sequences of Atg19 and p62 are unrelated to each other. Hydrophobic pockets conserved among Atg8 homologs recognized the side-chains of Trp and Leu in the WXXL motif in Atg19 and p62 (Figure 2). Mutational analyses of Atg8 and Atg19 showed that this interaction is indeed

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

required for the vacuolar transport of Ape1. Thus, we revealed the fundamental interaction between receptors and Atg8 homologs by which specific cargo molecules are efficiently captured by autophagosomes.

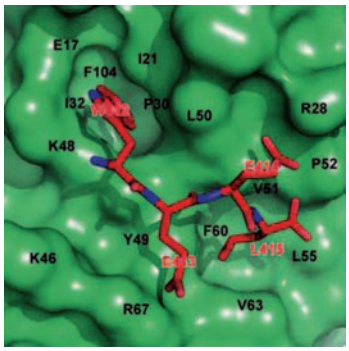

Figure 2. The structure of the interaction site of Atg8 with Atg19. The evolutionally conserved, hydrophobic binding pocket of Atg8 (surface model) accommodating a peptide corresponding to the Atg8-binding region of Atg19 (stick model) is shown.

Ⅳ. The determinants of substrate specificity in lipidation of Atg8

A ubiquitin-like system consisting of the E1 enzyme Atg7 and the E2 enzyme Atg3 mediates the conjugation of Atg8 with the lipid phosphatidylethanolamine (PE). The C-terminal carboxyl group of Atg8 is activated by Atg7, sequentially forms thioester intermediates with active site cysteine residues of Atg7 and Atg3, and eventually forms an amide bond with the amino group of PE. Probably as this lipid-modified form (Atg8–PE), Atg8 localizes to autophagosomal membranes and participates in their formation. The conjugation reaction of Atg8 can be reconstituted in vitro using purified proteins and liposomes containing PE. Whereas PE was identified as the sole in vivo target of Atg8, in vitro studies showed that the same system can mediate the conjugation of Atg8 with phosphatidylserine (PS) as efficiently as with PE. We found that, in contrast to PE conjugation, the PS conjugation of Atg8 is markedly suppressed at physiological pH (Figure 3). Furthermore, while the addition of acidic phospholipids to liposomes significantly promotes the production of Atg8–PE, this was not observed for Atg8–PS at all. Therefore, it was suggested that intracellular milieus, the pH of the cytosol and acidic phospholipids in membranes, contribute to the selective formation of Atg8–PE in the cell. We also showed that acidic phospholipids facilitate the binding of Atg8-Atg7 and Atg8-Atg3 thioester intermediates to the membrane (Figure 3).

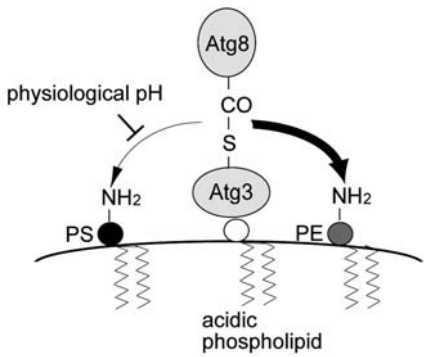

Figure 3. The neutral pH and the presence of acidic phospholipids result in the preferential formation of Atg8–PE.

Ⅴ. Amino-terminal region of Atg3 is essential for the association with PE in Atg8 lipidation

We previously showed using the in vitro system that the conjugate of the ubiquitin-like protein Atg12 with Atg5 (Atg12–Atg5) directly interacts with Atg3 and drastically stimulates its conjugating activity (transfer of Atg8 to PE). When PE-containing liposomes were absent from the reaction in the presence of Atg12–Atg5, Atg8 was mis-transferred to the serine residues in the N-terminal region of Atg3 from its active site cysteine, suggesting the involvement of the region in Atg8 lipidation. To examine the role of the N-terminal region of Atg3 in the formation of Atg8–PE, we prepared Atg3 mutants having deletions or mutations in this region. We found that the conjugating activities of the Atg3 mutants lacking N-terminal 7 amino acid residues or having the Leu-to-Asp mutation at position 6 were severely impaired both in vivo and in vitro. Furthermore, we revealed that the N-terminal region of Atg3 is responsible for the proper interaction with PE-containing membranes (Figure 4).

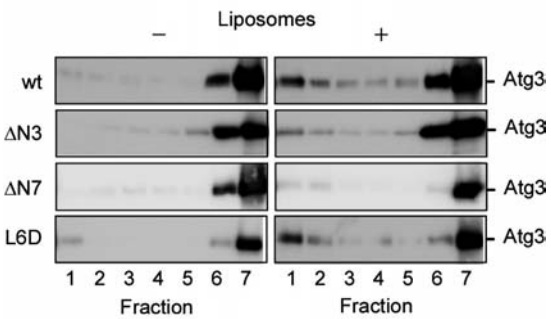

Figure 4. Floatation assay of Atg3 mutants. Purified Atg3 mutant proteins were incubated with liposomes containing PE, and then subjected to floatation assay. Fractions (fraction 1 represents the top fraction) were analyzed by western blotting with anti-Atg3 antibodies.

Ⅵ. The yeast Tor signaling pathway is involved in G2/M transition via Polo-kinase

The target of rapamycin (Tor) protein plays a central role in cell growth. Rapamycin inhibits cell growth and promotes cell cycle arrest at G1 (G0). Little is known, however, about whether or not Tor is involved in other stages of the cell division cycle. Unexpectedly, we found that the rapamycin-sensitive Tor complex 1 (TORC1) is involved in G2/M transition in *S. cerevisiae*. Strains carrying a temperature-sensitive allele of *KOG1* (*kog1-105*) encoding an essential component of TORC1, as well as yeast cells treated with rapamycin, show mitotic delay with prolonged G2 (Figure 5A). Overexpression of Cdc5, the yeast polo-like kinase,

rescues the growth defect of *kog1-105*, and in turn, Cdc5 activity is attenuated in *kog1-105* cells. The TORC1-Type2A phosphatase pathway mediates nucleocytoplasmic transport of Cdc5, which is a prerequisite for its proper localization and function (Figure 5B). The C-terminal polo-box domain of Cdc5 has an inhibitory role in nuclear translocation. Taken together, our results indicate a novel function of Tor in the regulation of cell cycle and proliferation.

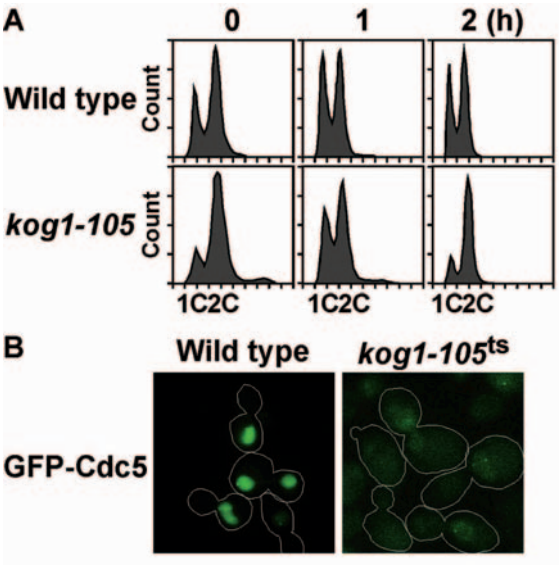

Figure 5. Tor regulates mitotic entry. (A) FACS analysis of wild type and *kog1-105*^{ts} strains. After temperature shift to 37°C, *kog1-105*^{ts} cells stop cell cycle progression with 2C DNA content, suggesting mitotic delay. (B) Localization of GFP-tagged Cdc5, the yeast polo-kinase. In wild type cells, Cdc5 localizes at the spindle pole body and the nucleus. In contrast, localization of Cdc5 is disrupted in the *kog1-105*^{ts} mutant.

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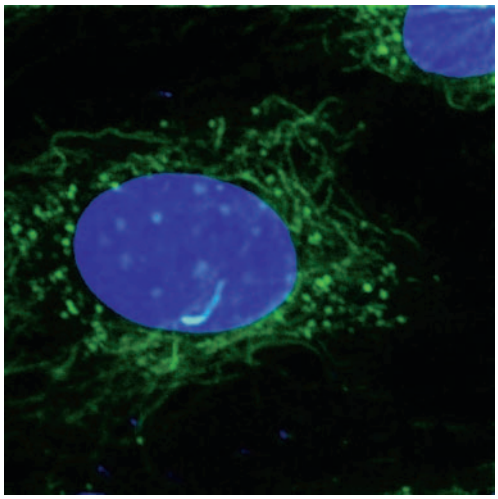
LABORATORY OF CELL STRUCTURE

Associate Professor
OGAWA, Kazuo

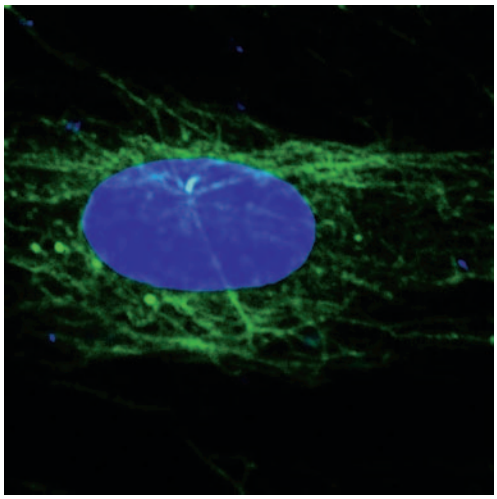
Numerous proteins localized in the cilia are linked to human diseases such as PCD (primary ciliary dyskinesia) and PCKD (polycystic kidney disease). Primary cilia are defined as single cilia that grow out of one of the centrioles during interphase in otherwise unciliated animal cells. They show a 9+0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

Such cilia can been 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.

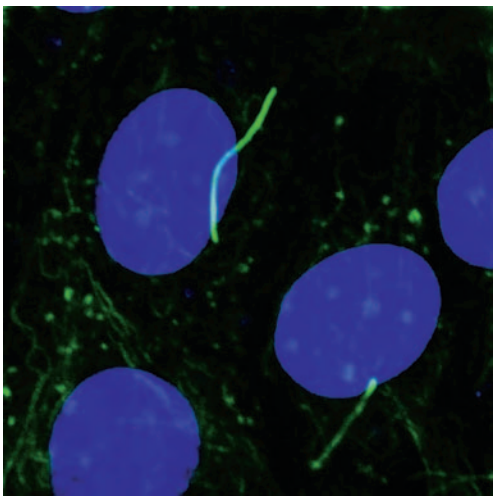
This year, four cell lines originating from adult mouse kidneys were established in order to study the proteomics of the primary cilia. The cell lines were named nibb-K1, K4, K5, and K8. The primary cilia of cells were observed by indirect immunofluorescence microscopy (Figure 1). In the cloned cells,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



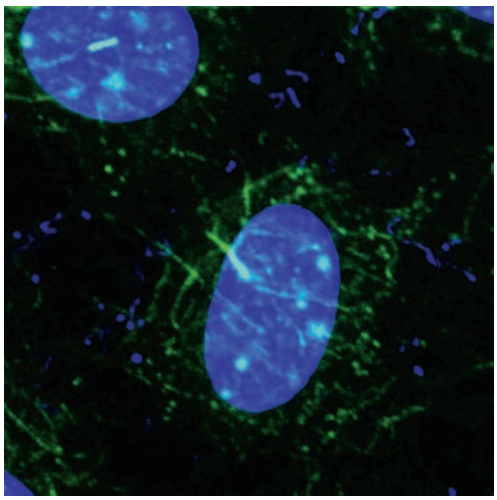
nibb-K1



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 Assistant GONDA, Naoko

Animal organs are made up of several types of cells, 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 decision by mediating cell-cell interactions, we are endeavouring 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 showed 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 appeared not to be drastically disturbed. Thus, in the developing mouse placenta, *Notch2* is likely not involved in cell fate decision, but rather participates in the formation of circulatory systems in the

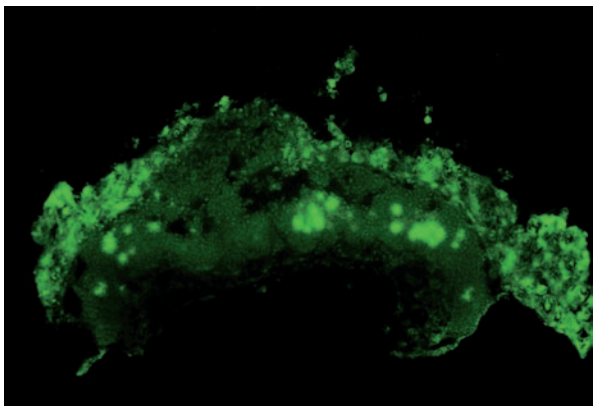


Figure 1. Cell death in *Notch2*$^{+/LacZ}$ mouse placenta at E8.5.
Acridine Orange (AO) positive signals were mostly observed in presumptive labyrinthine trophobast layer.

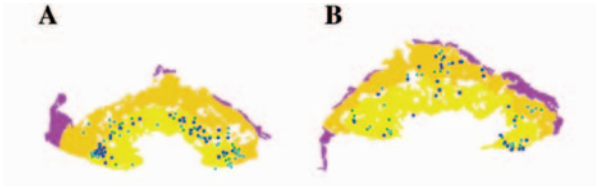


Figure 2. Distribution of AO positive signals in E9.5 *Notch2*$^{LacZ/LacZ}$ placenta (A) and *Notch2*$^{+/LacZ}$ placenta (B). Red, orange and yellow color indicates giant cell layer, spongiotrophoblast layer and labyrinthine trophoblast layer, respectively. Dots indicated in light blue, dark blue, and green show dying cells in three serial sections.

labyrinth layer where the expression of *Notch2* was 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 been unexplored. 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 by staining with a fluorescent dye, acridine orange, which has been employed to detect apoptotic cells in *Drosophila* (Figure 1). It was found that the appearance of dying cells correlated well with maternal vasculogenesis and was delayed in the mutant placenta (Figure 2). Identification of a factor which is responsible for the induction of *Notch2* expression in trophoblasts around newly forming maternal blood beds is another project in our laboratory. When both projects are accomplished, we should obtain some insights into *Notch2* gene function in maternal vasculogenesis in the developing mouse placenta.

Publication List

〔Original paper〕

● Tang, H., Brennan, J., Karl, J., Hamada, Y., Raetzman, L., and Capel, B. (2008). Notch signaling maintains Leydig progenitor cells in the mouse testis. Development *135*, 3745-3753.

DIVISION OF MORPHOGENESIS

Professor
UENO, Naoto

Associate Professor
KINOSHITA, Noriyuki

Assistant Professors	NAKAMURA, Makoto TAKAHASHI, Hiroki SUZUKI, Makoto
Technical Staff	TAKAGI, Chiyo
Postdoctoral Fellows	TAO, Hirotaka SHINDO, Asako
Graduate Students	MORITA, Hitoshi HARA, Yusuke
Technical Assistants	YAMAMOTO, Takamasa MURAKAMI, Michiyo
Secretaries	MIYAKE, Satoko TSUGE, Toyoko

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 understand 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, Wnt/PCP pathway is implicated.

To understand the role of one of the core components of the PCP pathway, we knocked out one of two *prickle*-related genes *mpk1* in mice. We found that the *mpk1*$^{-/-}$ mutants die in early embryogenesis between E5.5 and E6.5. The mutants showed 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. Furthermore, we showed *mpk1* genetically interacts with another PCP gene *Vangl2/stbm* in epiblast polarization, indicating that PCP pathway components *mpk1* and *Vangl2/stbm* are essential for early cell polarity, particularly the apical-basal polarity of the epiblast.

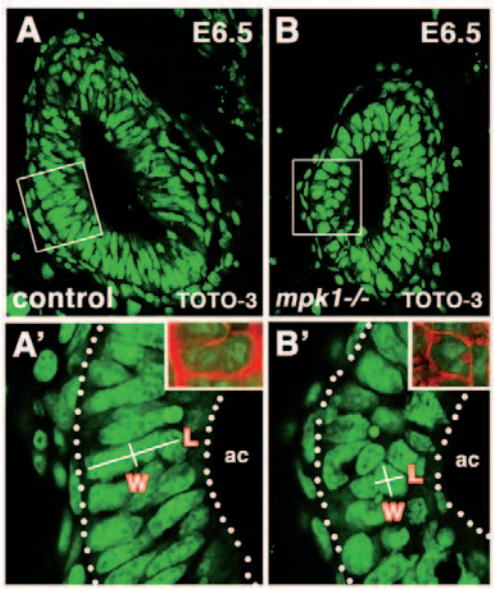


Figure 1. Fluorescent images of nuclei (stained by TOTO-3, green) in transverse sections of wild type (A, A’) and *mpk1*$^{-/-}$ mutant (B, B’) embryos. Insets are higher-magnification images of the respective epiblast cells indicated by F-actin (red) and nuclei (green). Arrowheads indicate the boundary between the extraembryonic and embryonic regions.

We also studied how PCP is established within the cells using explants of *Xenopus* embryonic tissues and found that heterogenous combination cultures of tissues such as mesoderm and ectoderm trigger the cell polarity as revealed by the live-imaging analysis of microtubule growth orientation. We are currently investigating whether physical force generated at the interface of two cell populations is involved in the initiation of the cell polarity.

Ⅱ. Protein ubiquitination and membrane trafficking involved in the Wnt/PC pathway

The Wnt/PCP signaling pathway has been shown to play an essential role in the regulation of gastrulation movements. However, the molecular mechanisms of how Wnt signals intracellularly and how it regulates the tissue movements remain elusive. In order to clarify the Wnt signal transduction mechanism, we searched for the proteins essential for this signaling pathway and implicated protein ubiquitination and membrane trafficking in the Wnt signal transduction. Dishevelled is a cytoplasmic protein, which plays a pivotal role in the Wnt pathway. When the Wnt pathway is activated, Dishevelled is translocated to the plasma membrane. This translocation activates downstream signaling components such as Rho GTPases, and regulates actin cytoskeleton and cell polarity. We have identified a novel E3 ubiquitin ligase complex that binds to Dishevelled.

This E3 ligase complex is essential for the translocation of Dishevelled. In addition, we have shown that Dishevelled and other PCP signaling components are ubiquitinated. This result suggests that protein ubiquitination is important for Wnt/PCP signal transduction. We also identified a novel SNARE family protein, xSynt2, which colocalizes with Dishevelled and is essential for its translocation to the plasma membrane. This SNARE protein is also shown to be essential for the gastrulation movements. SNARE family proteins are known to regulate membrane fusion processes. These findings show that the ubiquitin system and membrane trafficking regulate gastrulation movements in *Xenopus* embryos.

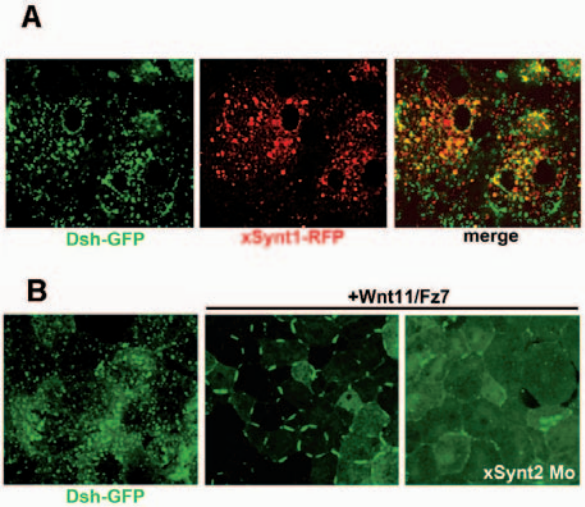


Figure 2. Membrane trafficking regulates the localization of Dishevelled. (A) Dishevelled shows punctate localization (green), and is colocalized with the SNARE protein xSynt1 (red). (B) Localization of Dshevelled-GFP (green). Wnt signaling translocates Dishevelled to the plasma membrane, which is inhibited by antisense Morpholino against xSynt2.

Ⅲ. Cellular morphogenesis during neural tube formation

Neural tube formation is one of the most dynamic morphogenetic processes during early embryogenesis and its failure is known to cause malformations known as neural tube defects. To understand cellular and molecular mechanisms regulating neural tube formation, we focused on MIDLINE1 (MID1) and a paralogous gene MID2. MID1 is a conserved microtubule-associated protein and responsible for Opitz G/BBB syndrome. This syndrome is associated with midline abnormalities such as hypertelorism, hypospadias, and heart defects, suggesting the developmental functions of MID1. We found that MID1 colocalized with microtubule in embryonic cells in *Xenopus*, and the combined depletion of MID1 and MID2 induced neural tube defects. Histological and live-imaging analyses also revealed that neuroepithelial cells in affected embryos became rounded and failed apical constriction and cell elongation. In addition, these defects are tightly correlated with the disorganization of the microtubule network along the apicobasal axis, which should be required for cell shape change and epithelial integrity. Theseese data suggest that the regulation of the microtubule network by MIDs is required for correct neural tube closure and that similar mechanisms also underlie the development of other organs affected in Opitz G/BBB syndrome patients.

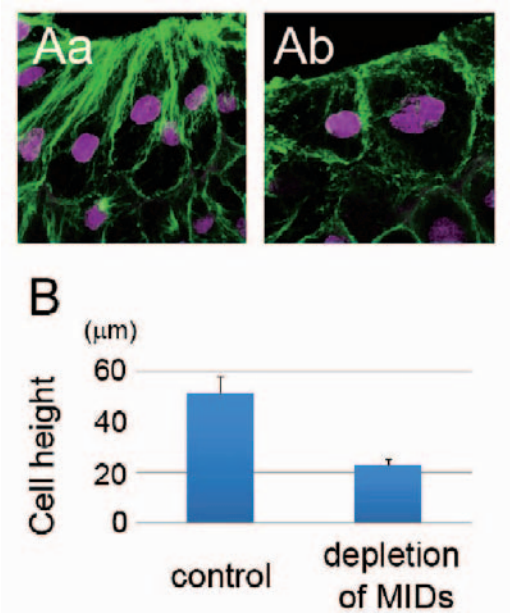


Figure 3. The changes of cell shape and the microtubule network during neural tube closure. (Aa) In control, neuroepithelial cells elongated their shape and developed microtubule along apicobasal axis (green). (Ab) In MIDs-depleted embryo, neuroepithelial cells became rounded and failed microtubule reorganization. (B) Quantitative data of cell height in superficial layer.

We also investigated the 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 embryo 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 the 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*

In vertebrates, *Brachyury*, a T-box transcription factor gene, seems to have a dual role in the differentiation of axial midline mesoderm cells into notochord and gastrulation cell movements regulated by non-canonical Wnt/planar cell polarity (Wnt/PCP) signaling. To annotate the function of *Brachyury*-downstream genes in chordate embryos, based on subtractive hybridization, dot-blot assays, EST sequences and the expression patterns in whole-mount *in situ* hybridization at embryonic stages, we developed a

knowledge database called "CINOBI: *Ciona* Notochord and *Brachyury*-downstream gene Index" to create comprehensive catalogues of *Brachyury*-downstream gene sets in *Ciona intestinalis*. Combining genome and large-scale cDNA data, we were able to characterize 450 non-redundant *Brachyury*-downstream genes. Twenty-four genes were newly annotated as notochord-expressed genes. Several genes are components of signaling pathways such as Wnt/PCP, NfkB and TGF-beta signaling. We propose that *Brachyury* is linked to these pathways, regulating the expression of each component, and that such a regulatory mechanism might be conserved among chordates.

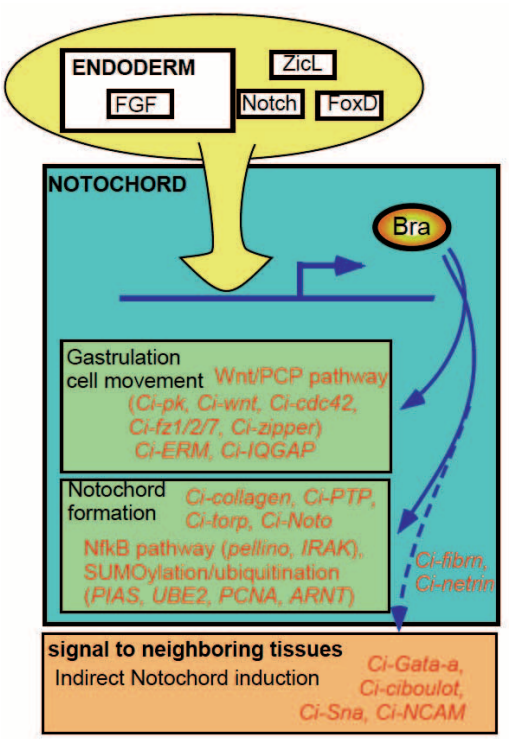


Figure 4. The role of *Brachyury* in the transcriptional network in *Ciona intestinalis*. Upstream positive regulators of *Brachyury* in the endoderm include *FGF* and *ZicL*. Brachyury directly activates *Ci-tropm* and other notochord-specific genes to induce differentiation of the notochord. Cell polarity-related genes such as *Ci-pk* and *Ci-Fz1/2/7* are involved in convergent extension movement in gastrulation. These signaling components are induced directly or indirectly by Brachyury.

Publication List

〔Original papers〕

- Shindo, A., Yamamoto, T.S. and Ueno, N. Coordonation of cell polarity during Xenopus Gastrulation. (2008). PLoS ONE *3*, e1600.
- Hotta, K., Takahashi, H., Satoh, N., and Gojobori, T. (2008). *Brachyury*-downstream gene sets in a chordate, *Ciona intestinalis*: Intrgrating notochord specification, morphogenesis and chordate evolution. Evo. Dev. *10*, 37-51.
- Kawasaki, A., Kumasaka, M., Satoh, A., Suzuki, M., Tamura, K., Goto, T., Asashima, M. and Yamamoto, H. (2008). Mitf contributes to melanosome distribution and melanophore dendricity. Pigment Cell Melanoma Res. *21*, 56-62.
- Gilchrist, M.J., Chiristensen, M.B., Harland, R., Pollet, N., Smith, J.C., Ueno, N. and Papalopulu, N. (2008). Evading the annotation bottleneck: using sequence similarity to search non-sequence gene data. BMC Bioinformatics *9*, 442.

DIVISION OF DEVELOPMENTAL GENETICS

Professor KOBAYASHI, Satoru

Assistant Professor	HAYASHI, Yoshiki
Technical Staff	NODA, Chiyo
NIBB Research Fellow	KUMATA, Yuji
PRESTO Researchers	SHIGENOBU, Shuji
	KAGEYAMA, Yuji
Postdoctoral Fellows	KITADATE, Yu
	HASHIMOTO, Yoshiko
	KONDO, Takefumi
Graduate Students	HAYASHI, Makoto
	YATSU, Jun
	MAEZAWA, Takanobu
	HASHIYAMA, Kazuya
Technical Assistant	SATO, Kaori
Secretary	HONDA, Satoko

Germ cells are the specialized cells that can transmit the 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 to form eggs and sperm when the organisms are physically matured.

Ⅰ. Maternal Nanos protein is required in pole cells to repress their apoptosis

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. In the absence of maternal Nos, pole cells undergo apoptosis during their migration to the embryonic gonads. Although Nos also plays important roles in repressing mitosis, somatic gene expression and somatic cell fate in pole cells, the primary role for Nos appears to be repressing apoptosis in the germline, because Nos is an evolutionarily conserved protein that is required for germline survival.

Apoptosis is mediated by caspases, a family of cysteine proteases that cleave diverse substrates to destroy cellular structure and integrity. Critical regulators of apoptosis function by antagonizing the inhibitor of apoptosis protein (IAP) that directly blocks caspase action. In *Drosophila*, four proapoptotic genes, *reaper* (*rpr*), *head involution defective* (*hid*), *grim* and *sickle* (*skl*) encode members of a family of related proteins that bind to and inactivate the IAP. They are also referred to as RHG genes.

We have demonstrate that maternal Nos represses apoptosis of pole cells by suppressing translation of *hid* RNA. In the absence of Nos activity, translation of *hid* mRNA yields a protein product that induces apoptosis. In addition, a maternally-provided protein kinase, Tao-1, is required to induce apoptosis in *nos* pole cells by promoting *skl* expression. Maternal *tao-1* RNA is enriched in the germ plasm and inherited by pole cells. Tao-1-dependent *skl* expression sensitizes pole cells to induction of apoptosis by *hid*. We propose that pole cells express *hid* and *skl* and become competent to undergo apoptosis during normal development. However, maternal Nos represses *hid* translation to inhibit apoptosis of pole cells for their survival.

We recently found that *hid* expression requires *eiger* (*egr*), a tumor necrosis factor (TNF) homolog, that is induced in pole cells by *decapetaplegic* (*dpp*). In addition, *p53* and *loki* (*lok*), a damage-activated kinase known to be required for phosphorylation of p53, are both required for hid expression in pole cells. Since maternal lok mRNA is enriched in germ plasm and then partitioned into pole cells, we speculated that ubiquitously distributed p53 may be activated in pole cells by maternal Lok. Taken together, we propose that *hid* expression is activated in a pole cell-specific manner by *loki/p53* and *dpp/egr* during normal embryogenesis (Figure 1).

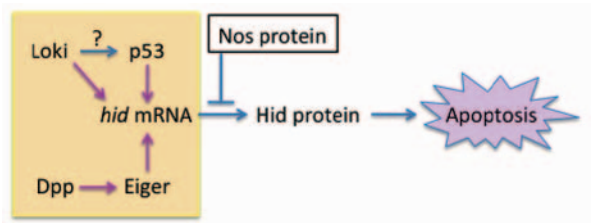


Figure 1. A model for the regulation of *hid* mRNA expression and apoptosis in pole cells.

Ⅱ. Expression of genes involved in sumoylation in pole cells

To identify the genes essential for germline development, we have performed EST and microarray analyses using pole cells and embryonic gonads. During the course of this analysis, we noticed that five genes in the SUMO (small ubiquitin-related modifier) conjugation pathway are highly expressed in pole cells and embryonic gonads (Hashiyama and Kobayashi, Gene Expression Patterns, *9*, 50-53, 2009).

Sumoylation regulates a wide range of cellular processes including transcription, genomic replication, nucleocytoplasmic signaling, and chromatin dynamics. SUMO proteins are covalently attached to lysine residues in the substrate protein by a series of enzymatic reactions similar to the ubiquitination system. Briefly, SUMO protein is processed by a SUMO-specific protease, and a SUMO-activating enzyme activates and transfers the SUMO protein to the SUMO-conjugating enzyme. SUMO is then covalently attached to the substrate protein by a SUMO protein ligase. We analyzed the spatiotemporal expression

patterns of the genes encoding the SUMO protein (*smt3*), the SUMO-specific protease (*Ulp1*), the SUMO-activating enzymes (*Uba2* and *Aos1*), and the SUMO-conjugating enzyme Ubc9 (*lesswright, lwr*) in *Drosophila* embryos and adult gonads (Figure 2).

Transcripts from all five genes are detected throughout the early embryo by whole mount in situ hybridization, while they are predominantly expressed in pole cells in late stage embryos. These genes are also expressed in the germline during oogenesis and spermatogenesis. We also found that SUMO protein is enriched in the nuclei of pole cells and gametogenic cells. Given that a large fraction of SUMO substrates are nuclear proteins, this data suggests that sumoylation is highly active in the germline. Our data provide a basis for understanding how sumoylation regulates germline development.

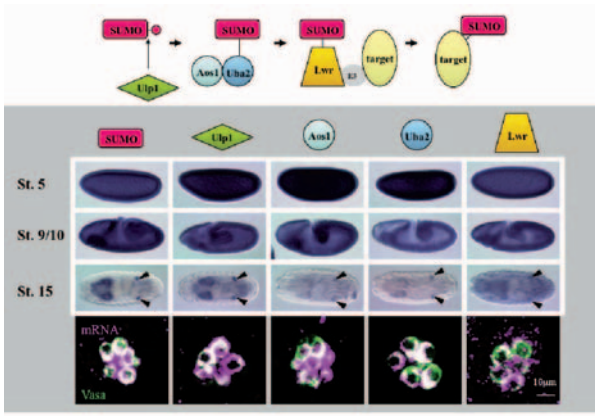


Figure 2. Expression of sumoylation genes in embryos.
In situ hybridization of embryos with antisense probes for *smt3* (sumo), *Ulp1*, *Uba2*, *Aos1* and *lwr*. Embryos at stage 5, stage 9/10 and stage 15 are shown. Anterior is to the left. Arrows indicate gonads.

Ⅲ. Mechanism leading to sexual dimorphism 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 (SGCs) to pole cells within the embryonic gonads. However, we 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. We also found that *Sex lethal* (*Sxl*) is expressed in the migrating pole cells in a female-specific manner, and its function is required for the feminization of pole cells. Ectopic expression of *Sxl* in XY (male) pole cells is able to direct the developmental fate of these cells to become functional eggs. Our results show that the germline-autonomous mechanism, along with the non-autonomous mechanism, leads to sexual dimorphism in the germline (Figure 3).

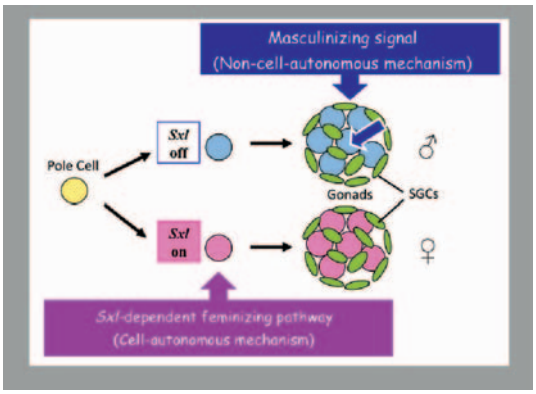


Figure 3. A model for the regulation of sexual dimorphism in pole cells.

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

The germline-stem-cell (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.

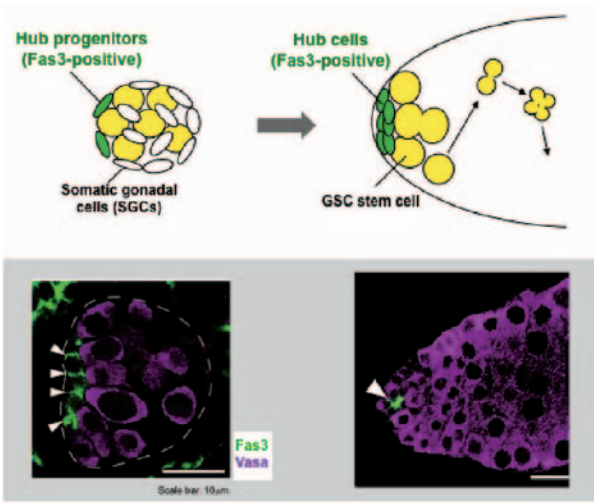


Figure 4. Hub and hub progenitors are marked with a molecular marker, Fasciclin3 (Fas3). Fas3 positive cells (green) are located in the anterior tips of both adult and embryonic gonads. Magenta shows germline cells.

Hub cells are derived from a subset of somatic gonadal cells (SGCs) that are located in the anterior region of male embryonic gonads (Figure 4). How the formation of hub progenitors is restricted in the anterior of embryonic gonads, however, remains elusive. We have demonstrated that a receptor tyrosine kinase, Sevenless (Sev), provides a cue to ensure that the hub cells develops in the anterior region of the 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.

Recently, we found that Egfr, like Sev, is activated by a ligand from pole cells to prevent hub differentiation in the posterior SGCs. We also showed that hub formation requires Notch, which is activated in almost all of the SGCs within the male embryonic gonads. Thus, we propose that almost all of the male SGCs become competent to form hub by the function of Notch, but niche formation is repressed in the posterior SGCs by Egfr and Sev, thereby restricting niche in the anterior region.

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

Stem cells possess the remarkable capacity to generate both daughter cells that retain a stem-cell identity and others that differentiate. Stem cells reside in dedicated cellular microenvironments termed niches. These niches dictate a stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. Recent studies have shown that several signaling pathways, such as TGF-beta and JAK/ STAT are essential for maintenance of the *Drosophila* GSCs. GSCs are surrounded by somatic cells (niche cells) which form the physical area of the niche and are responsible for signaling molecule secretion. Despite the importance of these molecules for GSC maintenance, it is not yet well understood how these secreted molecules can precisely define the region of GSC niche.

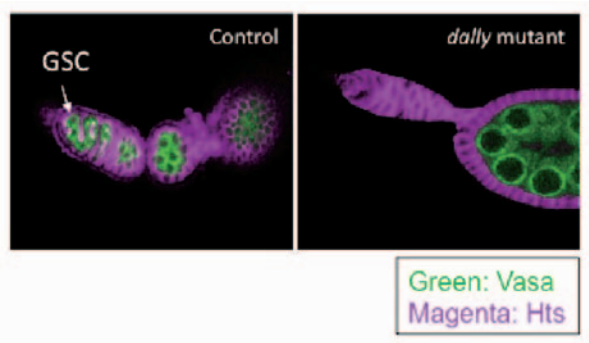


Figure 5. HSPG is essential for GSC maintenance in female gonads. In HSPG mutant (*dally*), GSCs are missing from the anterior tip of the ovary. Green and magenta show germline cells and somatic cells, respectively.

Heparan Sulfate Proteoglycans (HSPGs) are a glycoprotein 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 controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally*, can control the distribution of a TGF-beta ligand, Dpp, and establish Dpp gradient during wing development.

We showed that HSPGs are essential for GSC maintenance in both male and female gonads. In the HSPG mutant gonads, GSCs could not maintain their number and lost the characteristics of undifferentiated germ cells (Figure 5). In both male and female, HSPG genes are expressed in the GSC niche cells. We present the model that HSPGs have a role in defining the GSC niche by controling ligand stability or distribution in[the niche region.

Publication List

〔Original paper〕

- Yatsu, J., Hayashi, M., Mukai, M., Arita, K., Shigenobu, S., and Kobayashi, S. (2008). Identification of maternal RNAs encoding transcription factors required for germline-specific gene expression in *Drosophila* embryos. Int. J. Dev. Biol. *52*, 913-923.

DIVISION OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

Professor TAKADA, Shinji

Assistant Professor	OKUBO, Tadashi
Technical Staff	UTSUMI, Hideko
NIBB Research Fellow	AKANUMA, Takashi
Postdoctoral Fellows	TAKADA, Ritsuko
	CHEN, Qiuhong
	YABE, Taijiro
	NAKAYAMA, Kei
Graduate Students	ISHITANI, Shizuka
	TAKAHASHI, Jun
	TAKAHASHI, Hiroyuki
	ASANO, Takuya
Technical Assistants	TAKASHIRO, Kayoko
	ISHIKAWA, Mika
	TOMIDA, Sanai
	TSUZUKI, Chisa
Secretary	UKAI, Sakie

The body and tissues of the developing embryo are repeatedly divided into sub-regions specified by characteristic gene expression and morphology. The process that gives rise to these sub-regions according to a defined pattern is called “pattern formation” or “patterning.” 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 somite formation.

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. Recently, 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.

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 palmotoleoylation during embryogenesis using zebrafish embryos.

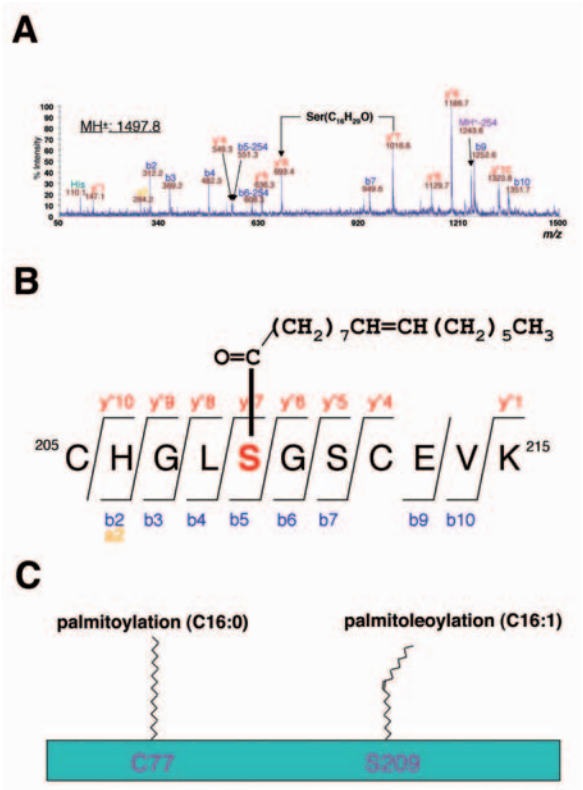


Figure 1. Lipid modifications of murine Wnt-3a protein (A, B) MASS spectrometric profile indicating modification with palmitoleic acid (C16:1) at Ser209, which is conserved among most Wnt proteins. (C) Schematic representation of lipid modification of murine Wnt-3a protein. Wnt-3a is modified with palmitolate (C16:1) at Ser209 and palmitate (C16:0) at Cys77. We found the palmitoleoylation is catalyzed by acytransferase, Porcupine (porc), in the ER. This modification is required for the trafficking of Wnt proteins from the ER.

Ⅱ. Characteristics of genes required for the development of somite or pharyngeal arches –Molecular mechanism of suppression by the Ripply family of transcriptional modulators–

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 boundary formation and mesenchymal-epithelial 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. Concomitant with the transition from the anterior PSM to somites, the characteristic gene expression in the PSM is translated into the segmental structure. However, most of the events accompanying the transition from the anterior PSM to somites have remained obscure.

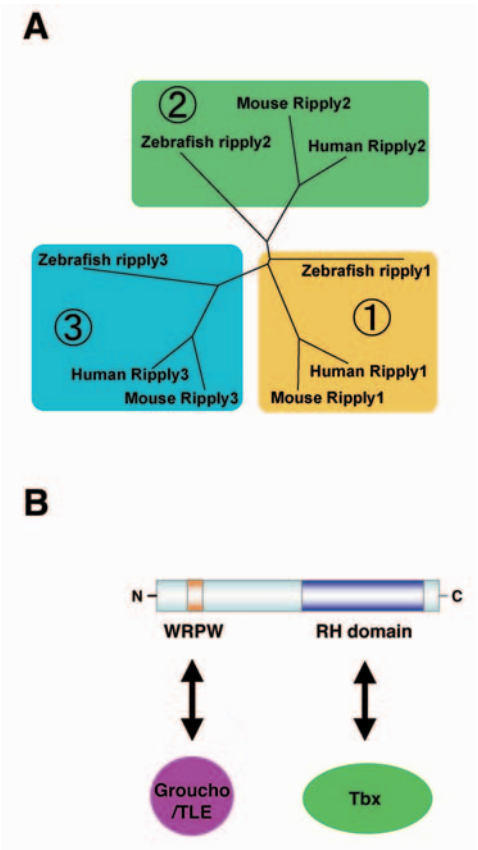


Figure 2. The Ripply family. (A) The phylogenic tree of the Ripply family. (B) Schematic structure of Ripply proteins.

We showed that a gene identified by our *in situ* hybridization screening, *ripply1*, encoding a nuclear protein associated with the transcriptional co-repressor Groucho, is required for this transition. Zebrafish *ripply1* is expressed in the anterior PSM and in several newly formed somites (Figure 2). Ripply1 represses specific gene expression in the PSM through a Groucho-interacting motif. In *ripply*1-deficient embryos, somite boundaries do not form, the characteristic gene expression in the PSM is not properly terminated, and the initially established rostrocaudal polarity in the segmental unit is not maintained, whereas paraxial mesoderm cells become differentiated. Thus, *ripply1* plays two key roles in the transition from the PSM to somites: termination of the segmentation program in the PSM, and maintenance of the rostrocaudal polarity.

In *ripply1*-deficient embryos, the expression of *mesp-b*, a key regulator in somite segmentation, is upregulated in a cell-autonomous manner, whereas, in embryos injected with *ripply1*mRNA, the expression of *mesp-b* is highly suppressed in the anterior PSM. These results suggest that Ripply1 regulates the proper expression of *mesp-b* in the anterior PSM.

Taking into account that the expression of *mesp-b* could be induced by T-box transcription factor (Tbx), we can speculate that Ripply1 may antagonize the function of Tbx in the transcription of *mesp* genes. The T-box family of transcription factors, defined by a conserved DNA binding domain called the T-box, regulates various aspects of embryogenesis by activating and/or repressing downstream genes. Reduced function of *T/Brachyury*, the founder member of this family, for example, causes truncated tail in the mouse. The importance of T-box transcription factors is also shown by the fact that mutations in human T-box genes cause severe congenital disorders, such as DiGeorge, Ulnar-mammary, and Holt-Oram syndromes. Furthermore, the T-box family is evolutionally conserved from C. *elegans* to insects and vertebrates, showing remarkable functional conservation across species. In spite of the biological significance of the T-box proteins, how they regulate transcription remains to be elucidated. Therefore, we carefully examined the relationship between Tbx and Ripply family proteins.

We showed that the Groucho/TLE-associated protein, Ripply, converts T-box proteins from activators to repressors (Figure 3). In cultured cells, zebrafish Ripply1, an essential component in somite segmentation, and its structural relatives, Ripply2 and Ripply3, suppress the transcriptional activation mediated by the T-box protein Tbx24, which is coexpressed with *ripply1* during segmentation. Ripply1 associates physically with Tbx24 and converts it to a repressor. Ripply1 also antagonizes the transcriptional activation of another T-box protein, No tail (Ntl), the zebrafish ortholog of Brachyury. Furthermore, injection of a high dosage of *ripply1* mRNA into zebrafish eggs causes defective development of the posterior trunk, similar to the phenotype observed in homozygous mutants of *ntl*. These results indicate that the intrinsic transcriptional property of T-box proteins is controlled by Ripply family proteins, which act as specific adaptors that recruit the global corepressor Groucho/TLE to T-box proteins.

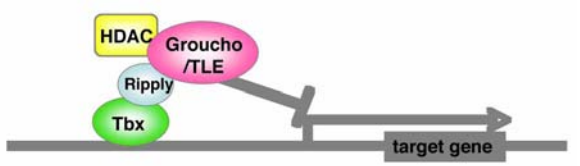


Figure 3. Transcriptional repression mediated by Ripply. Ripply converts transcriptional property of T-box transcription factor from activator to repressor by recruiting Groucho/TLE co-repressor complex.

To get further mechanistic insights into the interactions between Tbx24 and Ripply1, we next prepared several deletion or amino acid-substituted constructs of Tbx24 and Ripply1 (Figure 4A). By conducting immunoprecicipitation assays, we found that the T-domain alone, which is highly conserved among the Tbx family proteins, is sufficient for the association with Ripply1 but that another region of Tbx24 is dispensable for the interactions. On the other hand, a ~50 amino acid length sequence at the carboxyl terminus, called the Ripply homology domain, is conserved among the Ripply family proteins. Highly conserved sequences exist in this domain. Substitution of some of these sequences to an alanine stretch (Ripply1-mutFPVQ) results in a significant reduction in the transcriptional repression of the pBP-TbxX2 reporter construct (Figure 4B), indicating that this conserved sequence is required for Ripply-mediated transcriptional repression. This reduction appears to be due to decreased affinity of Ripply1-mutFPVQ for Tbx24 (Figure 4C). Consistent with this result, not only Ripply1 but also its structural relatives, Ripply2 and Ripply3, can suppress the transcriptional activation mediated by the T-box protein Tbx24 in cultured cells. Based on these results, many T-box proteins are likely to be converted from activators to repressors through specific interactions with Ripply proteins.

The identification of Ripply1 as a switching molecule for T-box genes suggests a novel transcriptional mechanism that could participate in other aspects of development, although the relationship between the Ripply family and T-box factors besides Tbx24 and Ntl remains to be elucidated. The overlapping expression of *ripply* and T-box genes, observed in various developing tissues and organs, supports the idea that the two proteins cooperate in development. Further understanding of the cooperative transcriptional regulation by Ripply and T-box proteins could help elucidate the mechanisms underlying the disproportionate activation or repression found in human genetic disorders associated with mutations in T-box genes.

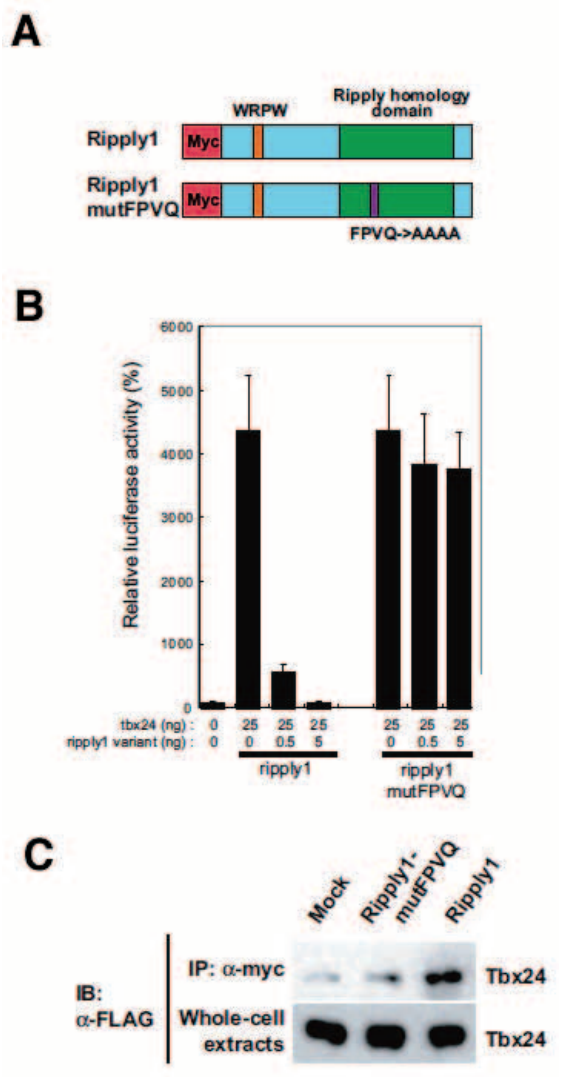


Figure 4. A conserved amino acid stretch in the Ripply homology domain required for interaction with T-box protein. (A) Schematic representation of a Ripply1 mutant used in the analysis shown in B and C. (B) *In vitro* reporter assay. (C) Immunoprecipitation to examine physical interaction between Rippply1 mutant and Tbx24.

Publication List

〔Original papers〕

- Alvarez-Medina, R., Cayuso, J., Okubo, T., Takada, S., and Marti, E. (2008). Wnt canonical pathway restricts graded Shh/Gli patterning activity through the regulation of Gli3 expression Development *135*, 237-247.
- Kawamura, A., Koshida, S., and Takada, S. (2008). Activator-to-repressor conversion of T-box transcription factors by the Ripply family of Groucho/TLE-associated mediators. Mol. Cell. Biol. *28*, 3236-3244.
- Shimizu, T., Kagawa, T., Inoue, T., Nonaka, A., Takada, S., Aburatani, H., and Taga, T. (2008). Stabilized β-catenin functions through TCF/LEF proteins and the Notch/RBP-J_κ complex to promote proliferation and suppress differentiation of neural precursor cells. Mol. Cell. Biol. *28*, 7427-7441.

DIVISION OF EMBYOLOGY

Professor FUJIMORI, Toshihiko

Assistant Professor TOYOOKA, Yayoi
NIBB Research Fellow KOMATSU, Kouji
Visiting Scientist SEKI, Touhaku

This laboratory was started in August 2008. The aim of our research is to understand the events underlying early mammalian development during the period from the pre-implantation to establishment of the body axes.

An understanding of early events during embryogenesis in mammals, as compared to other animals, has been relatively delayed. This is mainly due to the difficulties in the approaches to the developing embryos in the uterus of the mother. The other 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 discussion. 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. Our specific research interests are as follows.

Ⅰ. Characterization of the earliest event in body axis formation

It is generally accepted that, of the three body axes, the anterior-posterior (AP) axis is actively specified earliest. This is initiated by the movement of visceral endoderm from the distally located position to the future anterior side. It is still an open question whether any differential information relating to the future body axes already exists within the embryo before the movement of distally located visceral endoderm. We are planning to observe the behaviors of cells and the changes of embryonic morphology to characterize the earliest event of axis formation. We will analyze the general behaviors of cells within embryos by utilizing live imaging techniques in addition to morphological observation of embryos within the uterus.

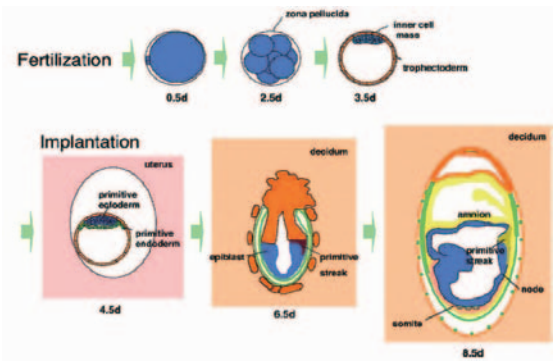


Figure 1. Summary of mouse early development.

Ⅱ. Molecular mechanisms regulating the cell differentiation in preimplantation development.

In early blastoycst, two types of cells - the cells of inner cell mass (ICM), and the cells of trophectoderm (TE) - can be distinguished. This is the earliest event of cell differentiation in mouse development. By the studies of genes expressed in the blastocyst and in the ES cells, several genes are known to play key roles in the regulation of cell differentiation during preimplantation stages. In the late blastocyst stage, expression of Oct4 is lost in TE and primitive endoderm (PE) cells. By contrast, Cdx2 is expressed in the TE lineage. Nanog is expressed in the ICM of early blastocyst and will be lost in PE lineage at a later stage. Continuous realtime analyses of the behaviors of these genes are unique, and will provide general basic ideas concerning the regulation of cell differentiation.

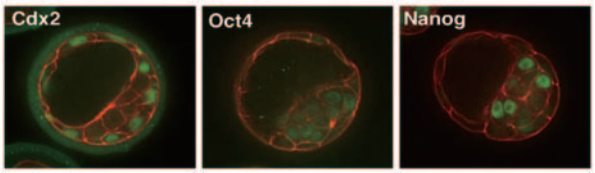


Figure 2. Expression of key genes in the mid blastocyst

Ⅲ. Analysis of mechanisms those regulate embryonic body shape

Mammalian early embryos change their total body size rapidly after the implantation, whereas the total shape of the embryo is well established. We will study the mechanisms regulating embryonic body shape by analyzing the behaviors of individual cells within the embryo.

Publication List

〔Original papers〕

- Ishii, Y., Matsumoto, Y., Watanabe, R., Elmi, M., Fujimori, T., Nissen, J., Cao, Y., Nabeshima, Y., Sasahara, M., and Funa, K. (2008). Characterization of neuroprogenitor cells expressing the PDGF beta-receptor within the subventricular zone of postnatal mice. Mol. Cell. Neurosci. *37*, 507-518.
- Katsuno, T., Umeda, K., Matsui, T., Hata, M., Tamura, A., Itoh, M., Takeuchi, K., Fujimori, T., Nabeshima, Y., Noda, T., Tsukita, S., and Tsukita, S. (2008). Deficiency of zonula occludens-1 causes embryonic lethal phenotype associated with defected yolk sac angiogenesis and apoptosis of embryonic cells. Mol. Biol. Cell *19*, 2465-2475.
- Tokunaga, A., Oya, T., Ishii, Y., Motomura, H., Nakamura, C., Ishizawa, S., Fujimori, T., Nabeshima, Y., Umezawa, A., Kanamori, M., Kimura, T., and Sasahara, M. (2008). PDGF receptor beta is a potent regulator of mesenchymal stromal cell function. J. Bone Miner. Res. *23*, 1519-1528.
- Toyooka, Y., Shimosato, D., Murakami, K., Takahashi, K., and Niwa, H. (2008). Identification and characterization of subpopulations in undifferentiated ES cell culture. Development *135*, 909-918.

DIVISION OF GERM CELL BIOLOGY

Professor
YOSHIDA, Shosei

Postdoctoral Fellow YOSHIDA, Naoko
Visiting Scientist SUGIMOTO, Ryo

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, as well as their niche, remains largely unknown in the context of homeostatic spermatogenesis. The Division of Germ Cell Biology, which was launched in 2008, aims to fully understand the mammalian spermatogenic stem cell system, mainly using the mouse system. Being a brand-new research division of NIBB, our overall research view and current foci of interests will be described. These include 1) the cellular nature of the stem cell compartment and their behaviors in the homeostatic spermatogenesis, and 2) the anatomical basis and function of the mammalian spermatogenic stem cell niche.

Ⅰ. Identifying the mouse spermatogenic stem cell compartments

1-1. Background: The mammalian spermatogenesis

Mammalian testes continuously produce numerous sperm during the reproduction period. 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 chains 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 pairs), and A_{al} ($A_{aligned}$; chains of 4, 8, 16 or occasionally 32 cells) contain stem cells.

Which fraction 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 so-called "A_s model" is currently most widely considered to be true. This model 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. This comprehensive model, however, is based upon "snapshots" of fixed specimens and involves too many theoretical limits to be entirely conclusive regarding stem cell function because "stem cells" by definition maintain themselves while producing differentiating progeny for a long period.

Transplantation of a single cell suspension into the recipient testis enables the functional and quantitative analyses of the "stem cells" based on their repopulating activity in the host testis. Combined with the morphology-based A_s model, it is often considered to be the case that all A_s spermatogonia are equivalent and act as stem cells, which support both homeostatic spermatogenesis and post-transplantation repopulation, despite the inherent limitation due to the lack of direct functional evidence.

1-2. Heterogeneous composition of the stem cells

In order to understand the nature of the mouse spermatogenic stem cell system in testicular tissue, we have been investigating the behavior and function of A_{undiff}. Authentic identification of A_{undiff} is based on morphology under electron or high-resolution light microscopy. Using the genetic labeling of A_{undiff} by means of the *Ngn3* regulatory sequence (Yoshida et. al., *Dev. Biol.* 2004), we have established experimental systems to investigate their live behaviors without disturbing normal tissue architecture.

We first asked whether the "stem cells" detected by transplantation are identical to the "stem cells" that actually self-renew in homeostasis, or whether these two "stem cells" represent different groups of cells. In order to address this question, a tamoxifen-dependent Cre recombinase (CreER^{TM}) was expressed in the Ngn3+ A_{undiff} (Yoshida et al., *Development* 2006). Together with an appropriate reporter transgene, this has enabled the irreversible pulse-labeling of A_{undiff} spermatogonia upon transient administration of tamoxifen, and the first quantitative detection of "actual stem cells" (i.e., the cells that persist for a long time while producing differentiating progeny, thus supporting tissue homeostasis). Intriguingly, the contribution of the pulse-labeled subpopulation of A_{undiff} to "actual stem cells" and "post-transplantation colony-forming stem cells" represents a great difference (approximately 40 times higher in the latter than in the former). Therefore, these two "stem cells" represent different subpopulations of A_{undiff}. We concluded that, in addition to actual stem cells, an extended population exists that does not self-renew while retaining the potential of self-renewal, which was defined as a population of "potential stem cells" (Nakagawa et al., *Dev. Cell* 2007).

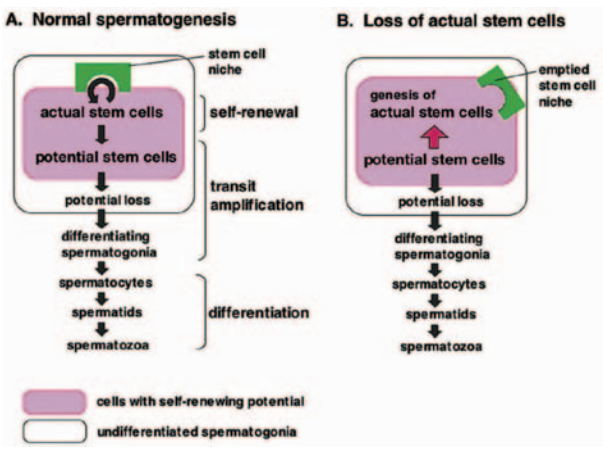


Figure 1. Proposed model of the functional compartments in the mouse spermatogenesis (Nakagawa et al., *Dev. Cell* 2007).

The "potential stem cells" were shown to rapidly turn over in homeostasis, suggesting that they consist of a transit-amplifying compartment.

Figure 1 shows our simplest interpretation of the hierarchical composition of the mouse spermatogenic stem cell system (Nakagawa et al., *Dev. Cell* 2007). In case of actual stem cell loss, potential stem cells might revert to the self-renewing mode and replenish actual stem cells. We are currently investigating the cell-biological characteristics of actual and potential stem cells.

Ⅱ. Testicular niche for A_{undiff}

2-1 Anatomy of the mouse testis and the research background

Spermatogenesis proceeds inside the seminiferous tubules, 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 seminiferous epithelium (the inner surface of the tubules). Therefore, in the 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.

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 is to clarify the niche of A_{undiff}.

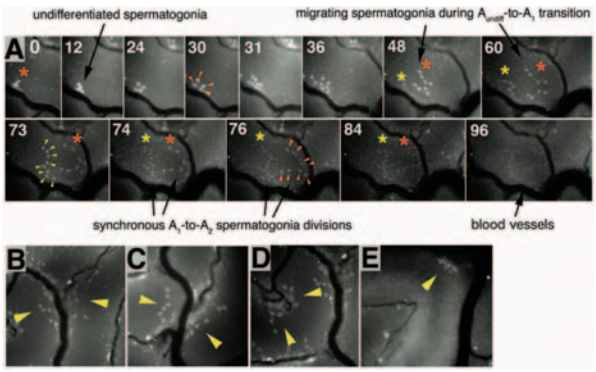


Figure 2. Localization of GFP-labeled Ngn3+ A_{undiff} and their relocation upon transition into differentiating spermatogonia (Yoshida et al., *Science* 2007).

(A) Live imaging of spermatogonia upon differentiation of A_{undiff}. Before differentiation (0 hour; the elapsed time indicated in each panel in hours), labeled A_{undiff} preferentially localized to the area adjacent to the blood vessels (seen as a black line) and surrounding interstitium. Upon differentiation, two chains of 8-cell cysts ($A_{al\text{-}8}$; indexed in yellow and orange) migrated from this position to spread all over the tubule (36-60h). Subsequently, the two cysts underwent synchronous mitotic division with 2-3 hours' interval, resulting in the formation of two 16-cell cysts of differentiating spermatogonia (73-74h).
(B-E) Examples of the vasculature-proximal localization of A_{undiff}. A_{undiff} (arrowhead) preferentially localized to areas adjacent to blood vessels, more characteristically to their branch points. In (B-C), A_{undiff} in neighboring seminiferous tubules show back-to-back localization over branching vessels.

2-2. Live imaging and the vasculature-associated niche for A_{undiff}

We have developed a live imaging system in which GFP-labeled Ngn3+ A_{undiff} and their progeny can be continuously filmed in undisturbed testes. It was revealed that A_{undiff} preferentially localize to the area adjacent to blood vessels and interstitial cells that surround the seminiferous tubules. In addition, the dynamic migration of spermatogonia from the vasculature proximity to spread throughout the tubules was also observed upon differentiation of A_{undiff} (Figure 2). The same relocation was also supported by three-dimensional reconstruction based on authentic morphological identification of A_{undiff} on serial sections (Figure 3). These extend the preceding observations from mouse and rat testis sections that A_{undiff} shows a significant biased localization to the interstitium, and we proposed such area as the niche for A_{undiff} (Yoshida et al., *Science* 2007).

We are currently investigating the cellular and molecular identification of this vasculature-associated niche.

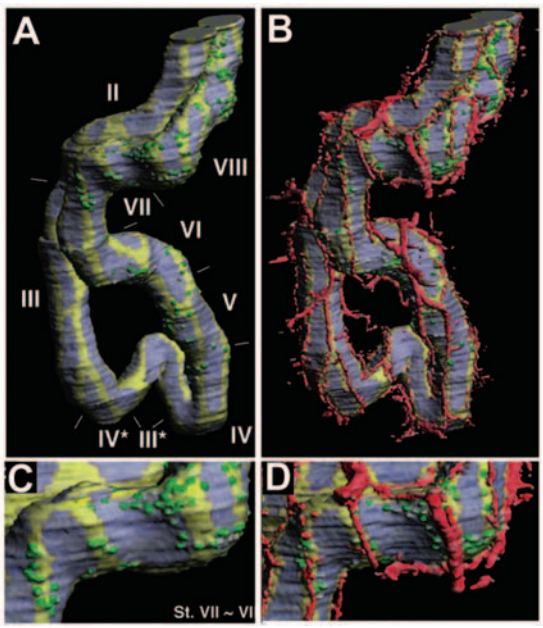


Figure 3. Localization of A_{undiff} revealed by three-dimensional reconstruction (Yoshida et al., *Science* 2007)

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.

Publication List

〔Original paper〕

- Sato, Y., Watanabe, T., Saito, D., Takahashi, T., Yoshida, S., Kohyama, J., Ohata, E., Okano, H., and Takahashi, Y. (2008). Notch mediates the segmental specification of angioblasts in somites and their directed migration toward the dorsal aorta in avian embryos. Dev. Cell. *14*, 890-901.

DIVISION OF REPRODUCTIVE BIOLOGY

Professor (Specially appointed)
NAGAHAMA, Yoshitaka

Professor	NAGAHAMA, Yoshitaka*
Assistant Professors	OKUBO, Kataaki
	OHNO, Kaoru
NIBB Research Fellow	SUZUKI, Aya
Postdoctoral Fellows	CUI, Jianzhou
	GUAN, Guijun
	LI, Jianzhong
	PAUL, Bindhu
	SAKAI, Fumie
	SHIBATA, Yasushi
	USAMI, Takeshi
	ZHOU, Linyan
Technical Assistants	HARA, Ikuyo
	SHIBATA, Emiko
	TAKAKI, Chikako
Secretary	SHIMADA, Yu

Fish exhibit a range of gonadal forms from gonochorism to several types of hermaphroditism, thus providing an excellent animal model to study the molecular mechanisms of sex determination/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 have been using medaka (*Oryzias latipes*) to investigate the molecular mechanisms of sex determination and Nile tilapia (*Oreochromis niloticus*) to investigate gonadal sex differentiation. In fish with a stable genetic XX/XY sex determining system, the sex-determining gene lies on the Y chromosome and is responsible for initiating male sex determination. We identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka, the first one in non-mammalian vertebrates. However, there is no sequence homology between the two known vertebrate sex-determining genes, *SRY/Sry* (mammals) and *DMY*. Another important difference is that *DMY* transgenic XX medaka are fully functional and fertile males, whereas *Sry* transgenic mice are sterile. 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. In medaka embryos, *gsdf* was predominantly expressed in the somatic cells in the XY gonads from the day of hatching. Conversely, expression of *gsdf* was found to be weaker in the XX gonads undergoing female sex differentiation. When the XY embryos were treated with estrogen, in order to reverse their phenotypic sex, a decline was observed in the expression of *gsdf* in those embryos. Treatment of the XX embryos with methytestosterone increased the expression of *gsdf*, proving that the expression of this gene is linked with the phenotypic sex, not the genetic sex.

In tilapia, all genetic female (XX) and male (XY) broods are available. Through cDNA subtraction between XX and XY gonads during sex differentiation and microarray hybridization followed by gene expression analyses by RT-PCR and *in situ* hybridization, we have concluded that the sex-specific expression of *Cyp19a1/Foxl2* in XX gonads and *DMRT1* in XY gonads during early gonadal differentiation (5 - 6 dph) is critical for indifferent gonads to differentiate into either the ovary or testis in the Nile tilapia. 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. It is of great interest to note that some of the sex reversed XX tilapia produced sperm with extremely high motility (Wang *et al*., unpublished).

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 swapping of the gonads is initiated through a switching in the expression of the gonadotropin hormone receptors, *FSHR* and *LHR*. These two genes act as mediators to convey the information about the change in social status to the to-be-active gonad. Most intriguingly, the sex change in these fish starts with a dramatic change in their sex-specific behavior immediately after they realize their social status. This suggests that the brain has a primary role in sexual development and plasticity and presents an interesting challenge for future researchers.

Ⅲ. Sexual plasticity in the adult gonochoristic fish

With the exception of certain hermaphroditic species, most vertebrate species are thought to have lost their sexual plasticity after differentiation of separate gonads/sexes with a single, distinct gamete type. Recently, we treated adult female tilapia with fadrozole (AI, a non-steroidal aromatase inhibitor) for two to five months to block the conversion of androgens to estrogens in order to investigate whether sexual plasticity is retained in the adult gonochoristic fish (Nakamura *et al*., unpublished). Suppression of estradiol-17β (E2) production via AI treatment caused a rapid degeneration of primary oocytes, leading to testicular germ cell differentiation in the adult ovary. Sex-changed fish show a typical male pattern of reproductive hormone levels and secondary sex characteristics, producing fertile sperms in the newly formed testes. Additionally, these fish display male-specific territorial behavior, pointing towards the changes that might have occurred to the sex-specific neuronal circuits in the brain. Conversely, co-treatment of E2 inhibited AI-induced sex reversal. Our results demonstrated for the first time in any gonochoristic species

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

that tilapia retains its sexual plasticity even in the adult stage. Furthermore, this data indicates that estrogens are vital to the maintenance of female phenotype in gonochoristic species.

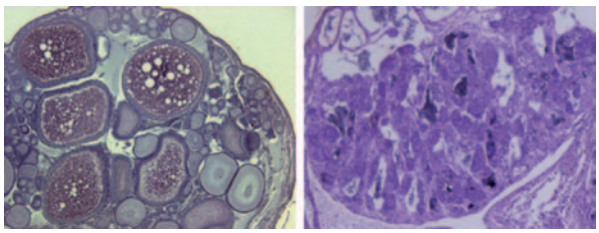


Figure 1. Aromatase inhibitor (AI)-induced female-to-male sex reversal of adult tilapia females. Left, Vehicle-treated gonad. Right, AI-treated gonad.

Ⅳ. Endocrine regulation of oocyte maturation and ovulation

A period of oocyte growth is followed by a process called oocyte maturation (the resumption of meiosis) which occurs prior to ovulation 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 (M. Mita, M. Yoshikuni *et al*., unpublished). Based on its cysteine motif, the purified GSS was classified as a member of the insulin/insulin-like growth factor (IGF)/relaxin superfamily. 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. *In situ* hybridization showed the transcription of GSS to occur in the periphery of radial nerves at the side of tube-feet. Thus, the current study represents the first evidence of a relaxin system in invertebrates and points towards a novel reproductive role for this peptide in starfish.

17α, 20β-DP has been shown to be involved in both oocyte maturation and ovulation (follicle rupture). Interestingly, these actions of 17α, 20β-DP are mediated through two different progestin receptors, the membrane (mPR) and nuclear (nPR) progestin receptors expressed in the oocyte surface and follicular granulosa cells, respectively. nPR transiently expresses after the timing of LH release. The expression of a protease, membrane-type matrix metalloproteinase 2 involved in follicle rupture rose after nPR expression by gonadotropin treatment. These results demonstrate that nPR induced by gonadotropin in granulosa cells may regulate the expression of factors involved in follicle rupture with 17α, 20β-DP as a ligand (Shibata *et al*., unpublished).

Publication List

〔Original papers〕

- Ijiri, S., Kaneko, H., Kobayashi, T., Wang, D.S., Sakai, F., Paul-Prasanth, B., Nakamura, M., and Nagahama, Y. (2008). Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol. Reprod. *78*, 333-341.
- Kobayashi, T., Kajiura-Kobayashi, H., Guan, G., and Nagahama, Y. (2008). Sexual dimorphic expression of DMRT1 and Sox9 during gonadal differentiation and hormone-induced sex reversal in the teleost fish Nile tilapia (*Oreochromis niloticus*). Dev. Dyn. *237*, 297-306.
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DIVISION FOR SEX DIFFERENTIATION

Professor (Concurrent)
MOROHASHI, Ken-ichirou

Assistant Professor	OGAWA, Hidesato
Technical Staff	OKA, Sanae
Postdoctoral Fellows	BABA, Takashi
	KOMATSU, Tomoko
	SHIMA, Yuichi
	ISHIMARU, Yoshiyasu
	MIYABAYASHI, Kanako
Graduate Students	KATSURA, Yukako
	SEKIHARA, Sanae
	KOUNO, Yukie
Visiting Scientists	KUSAKA, Masatomo
	NISHIYAMA, Aya
Technical Assistants	TOMIDA, Sanai
	HAYASHI, Tomoko

A number of genes are known to play crucial roles during gonadal differentiation. Some of them have been identified as the genes responsible for human diseases, while the functions of the other genes have been elucidated by the phenotypes of gene-disrupted mice. How the genes are regulated by upstream regulators, however, is not yet fully understood. Studies of this aspect of genes are quite important in order to define the molecular mechanisms mediating sex differentiation of the gonad.

Ⅰ. Fetal Leydig enhancer

During development of the testis, fetal and adult Leydig cells arise. The fetal mouse Leydig cells emerge at E12.5 and start to produce the male sex hormone androgen. The number of fetal Leydig cells decreases after birth. The adult Leydig cells start to develop at around postnatal day 10 and thereafter increase in number. The fetal and adult Leydig cells have been revealed to be morphologically and functionally different, and we have discussed whether these two populations originate from two distinct cells. The origins of the two Leydig cells, however, are still unclear.

We have studied the *Ad4BP/SF-1* gene locus by transgenic mouse assays and have recently identified the fetal Leydig cell-specific enhancer. Using the DNA fragment, a mouse line in which the lacZ reporter gene is driven was established. The lacZ expression was first recognized in the testicular interstitium at E12.5. The number of the lacZ-expressing cells increased during the fetal period and declined after birth. In the adult male mice, only a few lacZ-positive interstitial cells were detected. Immunohistochemical analyses revealed that the lacZ colocalized with Leydig cell-specific markers. These results indicated that the enhancer activity is strictly confined to the fetal Leydig cell population.

Ⅱ. Fetal to adult adrenal

As Leydig cells, adrenocortical cells synthesize steroid hormones. Moreover, the tissue is developmentally similar to Leydig cells in terms of the presence of fetal and adult cells. We isolated the fetal adrenal enhancer of the *Ad4BP/SF-1* gene and, using the enhancer, a transgenic mouse to drive Cre-recombinase was established. The mouse was crossed with Rosa 26 mice to trace the cell lineage of the fetal cells. Examination of the adult adrenal gland of the mice demonstrated that the adult adrenocortical cells are derived from the fetal adrenocortical cells. Further examination with another transgenic mouse carrying Cre-ER (a fusion protein of Cre and estrogen receptor, whose recombinase activity is induced in the presence of an estraogen antagonist) demonstrated that the early but not the late stage of the fetal adrenal cortex has the potential to differentiate into adult adrenal cortex.

This division was closed and moved to the Graduate School of Medical Sciences, Kyushu University in July, 2008. All the members of this division wish to thank the members of NIBB for the warm and friendly collaboration we enjoyed here. During the past 10 years at this institute, we recognized the importance of basic research and the responsibilities of the researchers. This experience has formed the backbone of our motivation as basic researchers.

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LABORATORY OF MOLECULAR GENETICS FOR REPRODUCTION

Associate Professor
TANAKA, Minoru

Associate Professor	TANAKA, Minoru
NIBB Research Fellows	NAKAMURA, Shuhei
	SAITO, Daisuke*
Postdoctoral Fellows	KOBAYASHI, Kayo
	SAITO, Daisuke
Visiting Scientists	AOKI, Yumiko
	NAKAMURA, Shuhei*
Technical Assistants	KINOSHITA, Chie
	NISHIMURA, Keiko
	WATAKABE, Ikuko
Secretary	YONEMITSU, Masako

Laboratory Scope

Our laboratory aims to reveal the molecular mechanisms of the formation of the gonads and sex differentiation. We use medaka fish (*Oryzias latipes*) for these purposes and have been generating transgenic medaka (Figure 1) enabling us to identify the 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 a defect in gonads and are performing a positional cloning. With these two unique analytical methods (visualising cells and mutants), we are attempting to unveil the fundamental mechanisms of sex differentiation and plasticity common to many organisms.

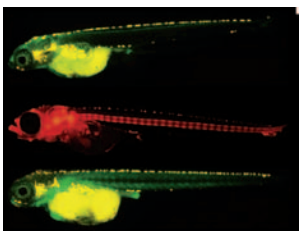

Figure 1. Various Transgenic medaka.

Ⅰ. Balancing between germ cells and gonadal somatic cells is important for sex differentiation of gonads.

We have generated medaka that completely lack germ cells in the gonad by impairment of PGC migration. These medaka reveal two important aspects of germ cell functions on the sex differentiation of gonads. Firstly, the morphological structure of germ cell-less gonads indicates the importance of germ cells for the formation of sex-specific structures in the gonads, possibly organizing the common unit into sex-specific structures dependent on its genetic sex. The second point is that germ cells are essential for development of the ovary. Without germ cells, both genetical female and male medaka exhibit male secondary sex characteristics and gonadal somatic cells are completely masculinized. These results suggest 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. The gene responsible for this phenotype is the type II receptor gene for anti-Müllerian homone (*amhrII*). We found that both *amhrII* and its ligand, *amh*, are expressed in gonadal somatic cells but not in germ cells.

In order to understand if male to female sex reversal in *hotei* mutants occurs because of a gonadal somatic cell-autonomous defect or because of a large number of germ cells, we have generated germ cell-deficient *hotei* mutants. None of the germ cell-less *hotei* mutants show any sex reversal of secondary sex characteristics. In addition, female-specific gene expression that can be seen in genetic male *hotei* mutants is also abolished when the germ cells are depleted. These results indicate that feminization of the gonad is a consequence of hypertrophic germ cells but not of a gonadal somatic cell-autonomous event.

The results from both germ cell-deficient medaka and germ cell-hypertrophic mutants (*hotei*) reveal 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 while gonadal somatic cells are predisposed to male development. According to this proposal, the function of the medaka testis determination gene on the Y chromosome can be explained as an enhancement of masculization that conquers canalization towards ovary by germ cells. The balancing of the two opposing characters, canalization of germ cells towards feminization and predisposition of gonadal somatic cells towards masculinization, may be a conserved cellular interaction among vertebrates. In fact, there have been several reports of the masculization of mice ovaries following the depletion of germ cells by genetic or physical manipulations. Even in an undifferentiated gonocolist, zebrafish, which firstly develops ovaries before some of the population turns into males, prior to the development of testis the germ cell number decreases once 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. in press).

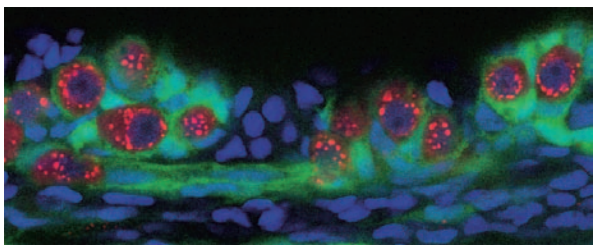

Figure 2. A right lobe of *sox9bp:GFP*-transgenic medaka. Blue: DAPI, Red: Germ Cell and Green: GFP.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

Ⅱ. Gonadal morphology during early stages of sex differentiation

Our previous results indicated that, prior to the formation of the gonadal primordium, two different precursors of gonadal somatic cells are established in the most posterior region of lateral plate mesoderm. These precursors express either *sox9b* or *ftz-f1*. In mammals, *sox9b* is specifically expressed in the male supporting cells surrounding germ cells (Sertoli cells and their precursors) and is known as the gene indispensible for testis formation. In order to understand the types of cells arising from the precursors in medaka gonad, we have generated transgenic medaka that allow us to keep track of *sox9b*-expressing cells in the gonad.

With the formation of the gonadal primordium, *sox9b*-expressing cells ensheathed primordial germ cells. Obervation of *sox9b*-expressing cells revealed the change of the morphology of the primordium. The gonadal primordium firstly forms as a single structure located along the midline. The cells that do not express *sox9b* appear and invade in the middle of the single gonadal primordium. As a consequence, the gonadal primordium splits into bilateral lobes. This process occurs soon after the single gonadal primorium forms (Figure 2).

Sox9b-expressing cells in male gonads keep surrounding the germ cells and begin to express the male supporting cell (Sertoli) marker, *dmrt1*, which is similar to those in mammalian testis development. In female gonads, the germ cells that undergo synchronous division enter meiosis and form follicles. Some of the population of germ cells in female gonads enters meiosis and follicles are formed. Unlike mammals, expression of *sox9b* is retained and found in the cells surrounding germ cells. Very interestingly, the granulosa cells of small follicles (primordial follicles) also express *sox9b*, but its expression diminishes immediately with the progress of folliculogenesis and the onset of the expression of the granulosa cell marker, *foxl2*.

Collectively, the observations using *sox9b*-transgenic medaka strongly suggest that the supporting cells expressing *sox9b* in the gonadal primordium are present as precursors of both Sertoli cells and granulosa cells.

Ⅲ. Germ cell development with change of the localization of germ-granule components

Germ granules are germ cell-specific intracellular structures and are essential for germ cell development. An examination of their components - olvas (vasa), nanos and tdrd1 (tudor) - reveals that they alter their localization in the cytoplasm during the early stage of sex differentiation of the gonads. By immunohistochemical analysis, these three germline-specific proteins were detectable on granule-like structures in the cytoplasm of migrating primordial germ cells (PGCs). In the germ cells of the gonadal primordia, these granules formed a hollow area lacking these three protein components. During the sexual differentiation of the female gonads, the granules were found to be reduced in size in the germ cells undergoing cystic division and they showed perinuclear localization in the oocytes. The germ cells in the male gonads, however, retained their hollow granules during this early sex differentiation stage. These results provide novel information on a distinct stage of germ cell development and suggest different stage-specific roles for germ granules (Figure 3).

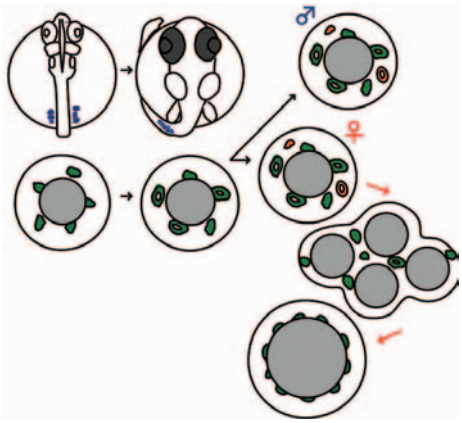

Figure 3. Change of localization of germ granule components olvas, nanos and tudor.

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LABORATORY OF PLANT ORGAN DEVELOPMENT

Director-General
OKADA, Kiyotaka

Assistant Professor	TATEMATSU, Kiyoshi
Postdoctoral Fellows	TSUCHIDA, Yuhei
	IGARASHI, Hisako
	URAWA, Hiroko
Visiting Scientists	WADA, Takuji
	TOMINAGA, Rumi
Graduate Students	TOYOKURA, Koichi
	IWASAKI, Akira
	TAMESHIGE, Toshiaki
Technical Assistant	HARA, Reiko
Secretary	SAKAGAMI, Mari

Plant organs - leaves, flowers, and roots - show impressive, symmetrical shapes, based on an ordered arrangement of differentiated cells. The organs are formed from a group of undifferentiated cells located at the tip of the stem or the root. In the case of leaves, the process of organogenesis starts with the formation of a leaf primordium in the peripheral zone of the shoot apical meristem 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 axis (central-marginal axis), and the adaxial-abaxial (foreside-backside) axis. In the course of proliferation and differentiation, the plant cells are believed to exchange information with neighboring or separated cells in order to regulate the organ architecture. We are trying to understand the mechanism of the information exchange between plant cells during the development of lateral organs such as leaves, sepals, petals, stamens and carpels.

Ⅰ. Genetic approach

Recent studies of Arabidopsis mutant show that a couple of 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. 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 action of microRNA165/166, which targeted the PHB, REV, and PHV messenger RNAs. Using laser microdissection techniques we also revealed that specific expression of miR165/166 genes in the abaxial side is important for the adaxial-abaxial boundary formation.

Ⅱ. Biochemcal approach

We are taking another approach to studying the intercellular signaling system by analyzing small peptides as candidates for intercellular signaling ligands, which are present in the apoplastic region of the shoot apical meristem (SAM).

Ⅲ. Microsurgical approach

We are also carrying out a microsurgical approach, ablating cells localized at SAM, at the peripheral zone, and at the leaf primodium, to investigate the cell-to-cell communication systems working in the leaf development by using a novel laser-ablating microscopy with UV-laser. When we ablated cells at SAM, the plant formed a filamentous leaf which lacked the adaxial-abaxial identity (Figure 1 bottom left).

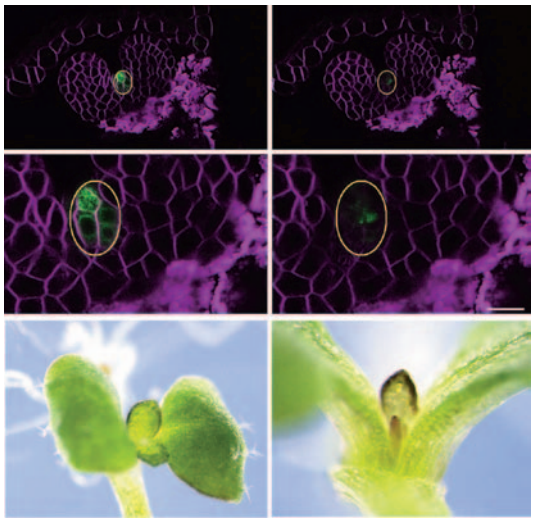

Figure 1. The disruption of cells at SAM by laser ablation system results in filamentous leaf formation. Top: Fluorescent images of SAM before (left) and after (right) the ablation. Middle: High magnification of the laser ablation point before (left) and after (right) ablation. Bottom: Leaf development of untreated control (left) and of the ablated plant (right). Laser ablated point is within the yellow circle. Scale bar: 10μm

Publication List

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- Sakai, T., Honing, van der H., Nishioka, M., Uehara, Y., Takahashi, M., Fujisawa, N., Saji, K., Seki, M., Shinozaki, K., Jones, M., Smirnoff, N., Okada, K., and Wasteneys, G. (2008). Armadillo repeat-containing kinesins and a NIMA-related kinase are required for epidermal cell morphogenesis in Arabidopsis. Plant J. *53*, 157-171.
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DIVISION OF MOLECULAR NEUROBIOLOGY

Professor
NODA, Masaharu

Assitant Professors	SHINTANI, Takafumi
	SAKUTA, Hiraki
	HIYAMA, Takeshi
Technical Staff	TAKEUCHI, Yasushi
NIBB Research Fellow	IHARA, Masaru
Postdoctoral Fellows	FUJIKAWA, Akihiro
	SUZUKI, Ryoko
	SHIMIZU, Hidetada
	NISHIHARA, Eri
	YOSHIDA, Masahide
	YONEHARA, Keisuke
Graduate Students	YONEHARA, Keisuke*
	CHOW, Pak Hong Jeremy
	NAGAKURA, Ayano
	ZHANG, Lanfan
	TANI, Sachiko
	SAKURABA, Jyuichi
Visiting Scientist	YONEHARA, Kayo
Technical Assistants	MIZOGUCHI, Masae
	KATAYAMA, Tomomi
	HATTORI, Nobuko
	TOMITA, Nao
	ISOSHIMA, Yoshiko
	MIURA, Seiko
Secretaries	KODAMA, Akiko
	YATAGAI, Hiroko

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, 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.

Fifteen years ago, we performed a large-scale screening using differential hybridization and restriction landmark cDNA scanning (RLCS) on the embryonic day 8 (E8) chick retina, and successfully identified 33 asymmetric molecules along the nasotemporal (N-T) axis and 20 along the dorsoventral (D-V) axis. We have almost revealed gene cascades of topographic molecules for retinal patterning and for the topographic retinotectal projection (see Annual Report 2006). We are now conducting experiments to elucidate the functional modes of CBF2, a winged-helix type transcription factor, which specifies the temporal character in the retina.

Ⅱ. Mechanisms for the topographic retinotectal projection

Special attention is now devoted to the molecular mechanisms for the 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*. We have obtained promising results indicating that our research will continue to shed light on these issues.

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 a 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. Previously, we reported a secretory protein, *SPIG1* (clone name; D/Bsp120I #1), preferentially expressed in the dorsal region in the developing chick retina (Shintani *et al*., 2004). Subsequently, we generated knock-in mice to visualize *SPIG1*-expressing cells with green fluorescent protein. We found that the mouse retina is subdivided into two distinct domains for *SPIG1* expression and *SPIG1* effectively marks a unique subtype of the retinal ganglion cells during the neonatal period (Figure 1).

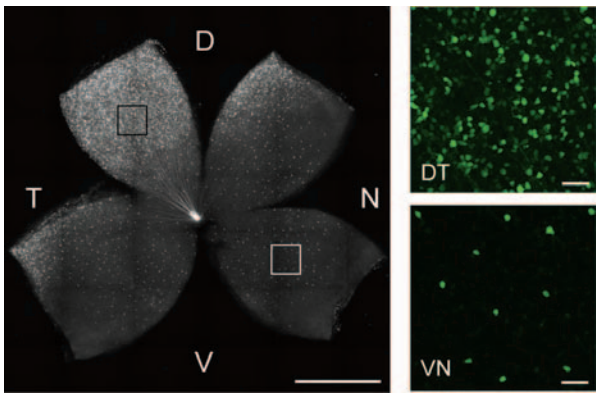

Figure 1. *SPIG1* expression in the mouse retina at P5. *SPIG1*-positive retinal ganglion cells are densely distributed in the dorsotemporal retina. In the remaining region of the retina, only a subtype of the retinal ganglion cell appears to express *SPIG1*, showing a mosaic distribution. Enlargements of the boxed regions are shown on the right. N, T, D, and V indicate nasal, temporal, dorsal, and ventral, respectively. Scale bars: 1 mm (left panel), 50μm (right panels).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

SPIG1-positive RGCs in the dorsotemporal domain project to the dorsal lateral geniculate nucleus (dLGN), superior colliculus, and accessory optic system (AOS). In contrast, in the remaining region, here named the pan-ventronasal domain, *SPIG1*-positive cells form a regular mosaic and project exclusively to the medial terminal nucleus (MTN) of the AOS that mediates the optokinetic nystagmus as early as P1. Their dendrites costratify with ON cholinergic amacrine strata in the inner plexiform layer as early as P3. These findings suggest that these *SPIG1*-positive cells are the ON direction selective ganglion cells (DSGCs). Moreover, the MTN-projecting cells in the pan-ventronasal domain are apparently composed of two distinct but interdependent regular mosaics depending on the presence or absence of *SPIG1*, indicating that they comprise two functionally distinct subtypes of the ON DSGCs. The formation of the regular mosaic appears to commence at the end of the prenatal stage and be completed through the peak period of the cell death at P6. SPIG1 will thus serve as a useful molecular marker for future studies on the development and function of ON DSGCs.

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.

4-1 Plasmin mediated processing of Ptprz

We identified the proteolytic processing of Ptprz by plasmin in the mouse brain, which is markedly enhanced after kainic acid (KA)-induced seizures. We mapped plasmin cleavage sites in the extracellular region of Ptprz by cell-based assays and *in vitro* digestion experiments with recombinant proteins (Figure 2). These findings indicate that Ptprz is a physiological target for activity-dependent proteolytic processing by the tPA/plasmin system, and suggest that the proteolytic cleavage is involved in the functional processes of the synapses during learning and memory.

4-2 Metalloproteinase- and γ-secretase-mediated processing of Ptprz

We also found that the extracellular region of the receptor isoforms of Ptprz are cleaved by metalloproteinases, and that the membrane-tethered fragment is subsequently cleaved by presenilin/γ-secretase, releasing its intracellular region into the cyto plasm (Figure 2). Notably, the intracellular fragment of Ptprz shows nuclear localization. Administration of GM6001 (an inhibitor of metalloproteinases) to mice demonstrated the metalloproteinase-mediated cleavage of Ptprz under physiological conditions. Furthermore, we identified the cleavage sites in the extracellular juxtamembrane region of Ptprz by tumor necrosis factor-a converting enzyme (TACE) and matrix metalloproteinase 9 (MMP-9). This is the first evidence of the metalloproteinase-mediated processing of a receptor-like protein-tyrosine phosphatase in the central nervous system.

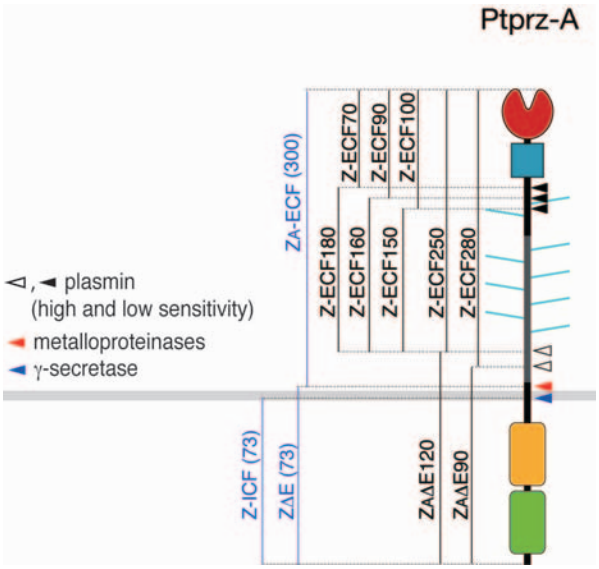

Figure 2. Proteolytic processing of Ptprz. Generated fragments are shown with their molecular size. Ptprz is a target for activity-dependent processing, which is supposedly involved in the synaptic remodeling during learning and memory.

4-3 Identification of TrkA as a substrate for Ptprz

Tropomyosin-related kinases (Trks) are single-pass transmembrane molecules that are highly expressed in the developing nervous system. Upon the ligand binding of neurotrophins, Trk receptors are activated through autophosphorylation of tyrosine residues; however, the PTPs responsible for the negative regulation of Trk receptors have not been fully elucidated. We identified Ptprz as a specific PTP that efficiently dephosphorylates TrkA as a substrate (Figure 3). Co-expression of Ptprz with Trk receptors in 293T cells showed that Ptprz suppresses the ligand-independent tyrosine phosphorylation of TrkA, but not of TrkB or TrkC, and that Ptprz attenuates TrkA activation induced by nerve growth factor (NGF). Co-expression analyses with TrkA mutants revealed that Ptprz dephosphorylates phosphotyrosine residues in the activation loop of the kinase domain, which are requisite for activation of the TrkA receptor. Consistent with these findings, forced expression of Ptprz in PC12D cells markedly inhibited neurite extension induced by a low dose of NGF. In addition, an increment in the tyrosine phosphorylation of TrkA was observed in the brain of *Ptprz*-deficient mice. Ptprz thus appears to be one of the PTPs which regulate the activation and signaling of TrkA receptors.

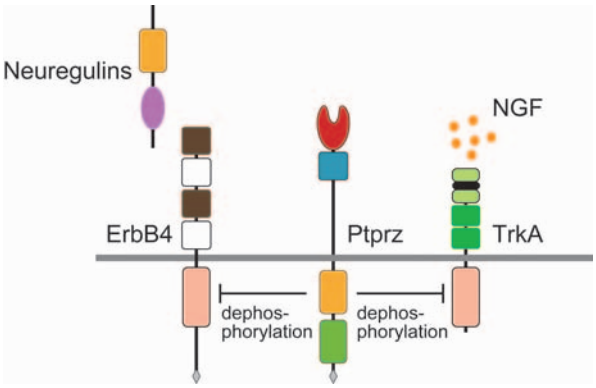

Figure 3. Schematic drawing of regulation of TrkA and ErbB4 by Ptprz. ErbB4 and TrkA are activated by their specific ligands, neuregulins and NGF, respectively. Ptprz attenuates the activation of ErbB4 (Fujikawa *et al*., 2007) and TrkA (Shintani *et al*., 2008) through dephosphorylation.

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

Na_x is exclusively localized to perineuronal lamellate processes extending from ependymal cells and astrocytes in the organs. Therefore, glial cells bearing Na_x channels are the first to sense a physiological increase in the level of sodium in body fluids. We revealed that Na-dependent activation of the metabolic state of the glial cells leads to extensive lactate production and that lactate stimulated the activity of GABAergic neurons in the subfornical organ (SFO). Thus, information of a physiological increase of the Na level in body fluids sensed by Na_x in glial cells is transmitted to neurons by lactate as a mediator to regulate the neural activity of the SFO to control salt-intake behavior.

Very recently, we found that Na_x-deficient mice show a reduced water intake compared with wild-type mice under non-feeding conditions. We suppose that animals deficient in the sodium-sensing system are apt to develop hypernatremia.

Publication List

〔Original papers〕

- Chow, J.P.H., Fujikawa, A., Shimizu, H., and Noda, M. (2008). Plasmin-mediated processing of protein tyrosine phosphatase receptor type Z in the mouse brain. Neurosci. Lett. *442*, 208-212.
- Chow, J.P.H., Fujikawa, A., Shimizu, H., Suzuki, R., and Noda, M. (2008). Metalloproteinase- and γ-secretase-mediated cleavage of protein tyrosine phosphatase receptor type Z. J. Biol. Chem. *283*, 30879-30889.
- Shintani, T., and Noda, M. (2008). Protein tyrosine phosphatase receptor type Z dephosphorylates TrkA receptors and attenuates NGF-dependent neurite outgrowth of PC12 cells. J. Biochem. *144*, 259-266.
- Tsuboi, N., Utsunomiya, T., Roberts, R.L., Ito, H., Takahashi, K., Noda, M., and Takahashi, T. (2008). The tyrosine phosphatase CD148 interacts with the p85 regulatory subunit of phosphoinositide 3-kinase. Biochem. J. *413*, 193-200.
- Yonehara, K., Shintani, T., Suzuki, R., Sakuta, H., Takeuchi, Y., Nakamura-Yonehara, K., and Noda, M. (2008). Expression of SPIG1 reveals development of a retinal ganglion cell subtype projecting to the medial terminal nucleus in the mouse. PLoS ONE *3*, e1533.

〔Review articles〕

- Noda, M., Takahashi, H., and Sakuta, H. (2008). Neural patterning: Eye fields. In Encyclopedia of Neuroscience, Squire, L.R., ed. (Elsevier), pp. 199-204.
- Sakuta, H., Suzuki, R., and Noda, M. (2008). Retrovirus vector-mediated gene transfer into the chick optic vesicle by *in ovo* electroporation. Dev. Growth Differ. *50*, 453-457.

DIVISION OF BRAIN BIOLOGY

Professor
YAMAMORI, Tetsuo

Assistant Professors	KOMINE, Yuriko
	WATAKABE, Akiya
	SADAKANE, Osamu
NIBB Research Fellows	TAKAHATA, Toru
	HIROKAWA, Junya
Technical Staff	OHSAWA, Sonoko
Postdoctoral Fellows	KOMATSU, Yusuke
Graduate Students	TAKAJI, Masafumi
	SASAKI, Tetsuya
	NAKAMURA, Tohru
	TOITA, Shigeko
	NAKAGAMI, Yuki
Technical Assistants	ISHIKAWA, Takako
	MORITA, Junko
	FURUYAMA, Makiko
	IMAI, Akiko

In order to understand the formation and evolution of the brain, we are studying the genes that are expressed in specific areas of the neocortex.

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

The neocortex emerged in mammals and evolved most remarkably in the primate. It is puzzling, however, that during mammalian evolution the neocortex was markedly expanded while the total number of genes in the mammal was little changed. In order to understand this puzzle, we studied gene expression patterns within different areas of the neocortex. In the last ten years, we reported the following findings, which are schematically illustrated in Figure 1.

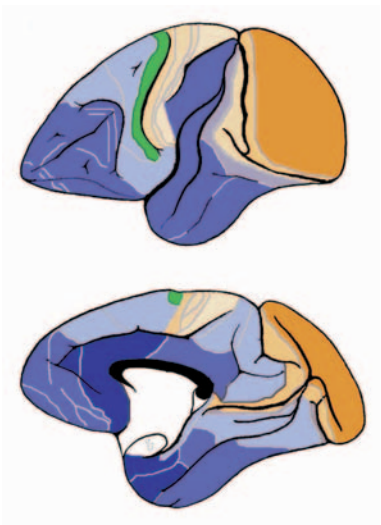

Figure 1. The expression of *occ1* (orange color) m *Rbp* (blue) and *gdf7* (green) are schematicaly illustrated in Brodmann's area figure in the guenon monkey. Top and bottom views are medial and lateral surfaces, respectively. (The figure is cited from Yamamori & Rockland, Neurosci. Res., *55*, 11-27, 2006).

By 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 screen area-specific molecules systematically in the monkey neocortex, we carried out a new 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. Among the spots that showed area difference, we cloned a gene that is specifically expressed in the visual area, which turned out to be the 5-HT1B receptor gene. (Watakabe et al., Cerebral Cortex, published online on December 4, 2008).

The expression of 5-HT1B revealed by in situ hybridization was strikingly high in V1 and the lateral geniculate nucleus (LGN) (Figure 2C, E). Because the mRNA expression was low in the extrastriate cortex, the abrupt change in the intensity of mRNA staining was observed at the border between V1 and V2 (Figure 2F).

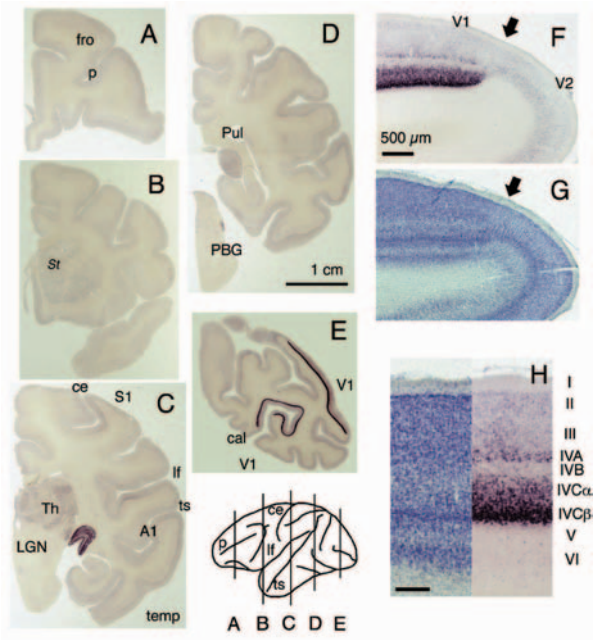

Figure 2. ISH analysis of 5-HT1B receptor mRNA. (A-E) Coronal sections of an adult monkey brain were prepared from the positions as depicted. Bar: 1 cm. Several areas are shown: fro, frontal area; temp, temporal area; S1, primary somatosensory area; A1, primary auditory area. PBG, parabigeminal nucleus; Pul, pulvinar nucleus; St, striatum. Major sulci are represented by lowercase letters: p, principal sulcus; cal, calcarin sulcus; ce, central sulcus; lf, lateral fissure; ts, superior temporal sulcus. (F) 5-HT1B receptor mRNA expression at the V1/V2 border (shown by the black arrow). Bar: 500 μm. (G) The adjacent Nissl-stained section. (H) 5-HT1B receptor mRNA expression in V1. Bar: 200 μm. (Watakabe et al., Cerebral Cortex, 2008)

Within V1, the expression of 5-HT1B receptor mRNA was mostly confined to layers IVA and IVC, the major geniculocortical input layers, and was particularly strong in the lower part of layer IVC beta (Figure 2H). In addition, the expression was also observed at lower intensity in layers II/III and VI of V1, which also receive geniculocortical inputs to a lesser extent. In the LGN, the strong mRNA expression was observed in all 6 layers, and there was no significant difference in staining intensity between the magnocellular and parvocellular layers. The 5-HT1B receptor mRNA expression was by far the most conspicuous in V1, followed by that in the LGN.

Among the 13 genes of serotonin receptors, 5-HT2A receptor mRNA exhibited area and lamina preferences similar to those of 5-HT1B receptor mRNA, although its expression was moderate across all areas. Both mRNA species were highly concentrated in the geniculorecipient layers IVA and IVC, where they were coexpressed in the same neurons. Monocular inactivation by tetrodotoxin injection resulted in a strong and rapid (<3 h) downregulation of these mRNAs, suggesting the retinal activity dependency of their expression.

In collaboration with Professor Hiromichi Sato's laboratory (Osaka University), we examined the roles of the two serotonin receptors. The activation of 5-HT1B receptors in V1 by specific agonist generally facilitates visual responses but tends to suppress weak responses. Our analysis suggests that, in geniculocortical transmission, nonsychronized spontaneous activity (noise) from the LGN neurons would be reduced by the suppressive effect of 5-HT1B receptors, but the visually evoked synchronized signals would be preserved or efficiently transferred to V1, thus, enhancing the S/N ratio in input-output relationship. On the other hand, neurons in the input layers of V1 which abundantly express the 5-HT2A receptor may act as a gain controller by enhancing weak signal response and suppressing excessive response. We therefore suggest that serotonin release in V1 exerts coordinated modulatory effects through 5-HT1B and 5-HT2A receptors on the V1 neurons. It is therefore possible that the serotonin system has contributed to the evolution of the elaborated function of the primate visual system.

The other gene is testican-1, which is specifically expressed in the primate visual cortex. Since testican-1 is a member of a testican family gene which also includes occ1, we were particularly interested in examining all the known members of the testican family (testican-1, testican-2, testican-3, SPARC, SC1) other than occ1 (Takahata et al., Cerebral Cortex, published online on December 10, 2008).

Interestingly, the in situ hybridization revealed that there are three groups of expression pattern among the occ1-related (testican) family members. The expression patterns of testican-1 and testican-2 are similar to that of occ-1, starting high level in V1, progressively decreasing along the ventral visual pathway, and ending low level in the temporal areas. Complementary to them, the neuronal expression of *SPARC* mRNA is abundant in the association areas and scarce in V1. Therefore, the expression pattern of *SPARC* is similar to that of *Rbp*. In addition, whereas *occ1*, *testican-1*, and *testican-2* mRNAs are preferentially distributed in thalamorecipient layers including "blobs," *SPARC* mRNA expression avoids these layers. Neither *SC1* nor *testican-3* mRNA expression is selective to particular areas, but *SC1* mRNA is abundantly observed in blobs.

Lines of evidence suggest that ECM considered to modulate neuronal development and plastic changes. Several groups have proposed that ECM limits plasticity in the rodent neocortex and ECM degeneration is required to implement ocular dominance plasticity (Pizzorusso et al. 2002; Oray et al. 2004). Secreted glycoproteins, such as Reelin, regulate both neuronal positioning in the developing nervous system and synaptic plasticity in the adult (Bock and Herz 2003; Dityatev and Schachner 2006). The area selectivity and activity dependence in expression of occ1-related genes, secreted glycoproteins, raise the possibility that OCC1-related proteins modulate synaptic plasticity in the adult cerebral cortex, some of which mechanisms are specific in primates.

Ⅱ. Gene expression under multisensory enhancement

We have been collaborating with professor Yoshio Sakurai (Kyoto University) and developed an audio-visual discrimination task (AVD-task) system, placing auditory and visual stimuli in the same position. We use nose-poking to measure the reaction time in which a rat responds to stimuli. Using this behavioral system, we were able to confirm amodal recognition of space, which means that a rat can respond to a different modality (visual or auditory) if the stimuli are in the same position, as previously reported in other systems. We also confirmed multisensory enhancement is indeed observed in rats. These results suggest that this new modified AVD system can be used to explore the molecular and cellular mechanisms underlying multisensory processing in rats (Sakata *et al*. Exp. Brain Res., *159*, 409-417, 2004).

We studied multisensory processing further using a c-Fos mapping technique. We first developed a method to standardize the cortex to quantitatively evaluate c-Fos expression by an automatic image analyzing system (Figure 3).

Combining this system with our newly developed "Cortical Box Methods" (Figure 3), we found the secondary visual cortex (V2L) in rats is specifically activated under audiovisual multisensory stimulation. Injecting muscimol into V1, V2, V2L and superior colliculus (SC), we found that V2L is specifically involved in the stimulation of multisensory reaction (Hirokawa *et al*., 2008).

Traditionally, multisensory integration was thought to occur in higher neocortical areas through a merging of different modalities of primary sensory information. Our results suggest that the multisensory integration may in fact occur at a relatively "early sensory" area such as V2. Previous elecrophysiological studies also show that there exist multisensory areas in the secondary visual area and in the boundary areas between two modal areas (Toldi *et al*., 1986; Barth *et al*., 1995; Wallace *et al*., 2004). This observation is consistent with our findings using the newly developed behavioral system and c-Fos analyzing system.

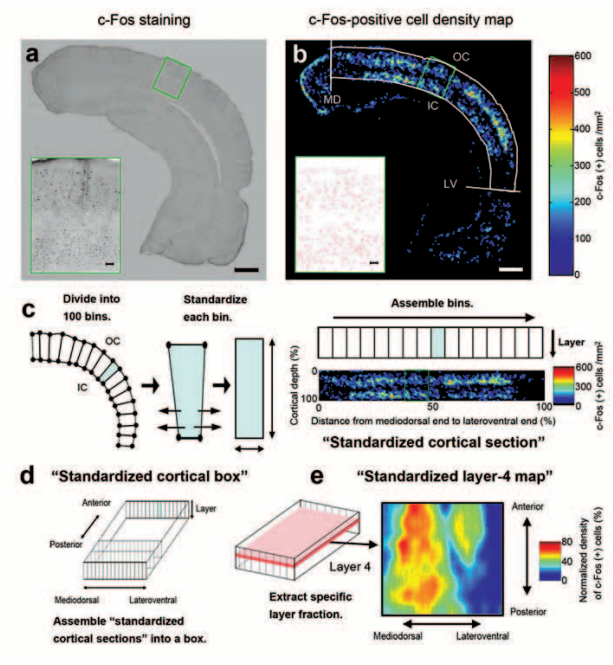

Figure 3 (a) Representative photomicrograph of cortical section stained by antibody against c-Fos protein. (b) The local density of c-Fos-positive cells was computed and pseudocolored. The mediodorsal end (MD), lateroventral end (LV), inner contour (IC) and outer contour (OC) were manually chosen to extract part of the cortex. Scale bars, 1 mm (a, b) and 100 mm (insets in a, b). (c) The extracted cortex was divided into 100 bins (left), and each bin was converted into a standard rectangle (left to center as shown by thick arrows). The standardized rectangular bins were reassembled into a stripe to form a standardized cortical section (right). (d) Standardized cortical sections were assembled from the posterior section to the anterior section to form a standardized cortical box. (e) A specific layer fraction (layer 4, for example) from a standardized cortical box was extracted to construct a standardized layer map (Shown in Hirokawa et al., 2008).

Ⅲ. Neocortical areas revealed by layer specific gene expression in rats.

To investigate area differences in rodent neocortex in terms of gene expressions, we chose three genes, *RORbeta, ER81* and *Nurr1*, whose mRNAs are mainly expressed in layers 4, 5 and 6, respectively. To reveal their complex spatial distribution patterns, we used double in situ hybridization histochemistry (ISH) and cortical box method for the analyses, as mentioned above (Hirokawa et al., 2008).

Double ISH revealed the large area differences in the relative abundance and extent of intermixing of the expressions of these mRNAs. Based on this finding, we quantitatively analyzed the ISH patterns of the three genes by cortical box method as described above. We made three major discoveries. Firstly, the three genes showed unique area distribution patterns that were mostly complementary to one another. Secondly, the cortical areas defined on the basis of the expression patterns of the three genes matched well with the cytoarchitectonic areas defined by Nissl staining. Finally, principal component analysis results suggested that the expressions of these three genes may be dictated by common rules that are tightly associated with the function and topology of the cortical areas.

The expression of the three layer specific genes showed tight relationships with the functional areas. This not only provides insight into the cortical area architecture underlining the complex spatial expression patterns of these genes, but also may serve as a framework for investigating gene expression regulation within the neocortex for future researchers.

Publication List

〔Original papers〕

- Hirokawa, J., Watakabe, A., Ohsawa, S., and Yamamori, T. (2008). Analysis of Area-Specific Expression Patterns of RORbeta, ER81 and Nurr1 mRNAs in Rat Neocortex by Double In Situ Hybridization and Cortical Box Method. PLoS ONE *3*, e3266.
- Lyckman, A.W., Horng, S., Leamey, C.A., Tropea, D., Watakabe, A., Van Wart, A., McCurry, C., Yamamori, T., and Sur, M. (2008). Gene expression patterns in visual cortex during the critical period: Synaptic stabilization and reversal by visual deprivation. Proc. Natl. Acad. Sci. USA *105*, 9409-9414.
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- Takahata, T., Hashikawa, T., Higo, N., Tochitani, S., and Yamamori, T. (2008). Difference in sensory dependence of occ1/Follistatin-related protein expression between macaques and mice. J. Chem. Neuroanat. *35*, 146-157.

LABORATORY OF NEUROPHYSIOLOGY

Associate Professor
WATANABE, Eiji

NIBB Research Fellow MATSUNAGA, Wataru

Animals respond to the physical and chemical stimuli of their environment. Most higher vertebrates have evolved a highly developed visual system to respond to light. “Why can we see?” This question is a fundamental one for a thorough understanding of these animals, including human beings. In order to more completely understand the sensory systems of animals, we decided to move ahead to research of visual systems this year, though we had been researching the salt-sensing system (~March, 2008). The research objects of our new project were the medaka fish and human beings. Various aspects of their behaviors rely on their visual systems.

Ⅰ. Psychobiological studies of Medaka

One of our major subjects is the psychobiological study of medaka (*Oryzias latipes*). Medaka is a world-wide teleost fish model, as is zebrafish (*Danio rerio*). Important discoveries (including advances in the areas of heredity of body colour and the sex-determning gene) were brought about through developmental studies of medaka. Medaka is not widely used, however, in neurobiological fields, and standardized methods of behavioral phenotyping for medaka have been not established.

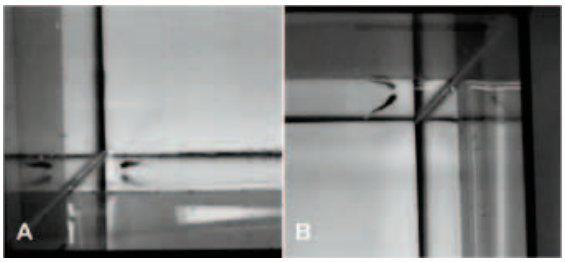

Figure 1. Representative examples of Medaka fish behaviors. A) Diving response. When Medaka fish are transferred to a novel environment, they immediately move to the bottom of the water tank. B) Climbing response. Medaka move to the water surface within several minutes.

The brain structure of medaka meets the minimum requirements for simulating the human brain. For neurobiological or behavioral studies, small teleosts (including medaka) are sometimes more useful than rodents thanks to their simplicity. Presently, zebrafish is more popular as a model animal than medaka. Medaka, however, has unique behavioral characteristics. Medaka can swim backward and hover within water flow by doing so. Moreover, medaka show interesting optomotor responses such as the dorsal light response under microgravity and the maintenance of their relative position to a rotating stripes pattern around them. Medaka, therefore, is a noteworthy model for the study of brain and behavior.

Our first goal is finding the medaka's behavioral characteristics that are applicable to the behavioral phenotyping of mutants and the study of relations between brain and behavior. We are attempting to classify typical normal behaviors of medaka (Figure 1). We are also studying the effects of acute stimulations by psychoactive drugs such as ethanol, nicotine and caffeine on medaka. These attempts will enable us to precisely describe the behaviors of medaka, which will form the basis of our future studies.

Ⅱ. Psychobiological studies of Human

Another of our major subjects is the psychobiological study of human beings (*Homo sapiens*). The visual system of an animal can be correctly understood by comparing two or more animals. Therefore, in our laboratory we address the issue of the human visual system in tandem with that of medaka. Recently, it has become possible to make complex visual stimuli easily by using computers and digital display systems, and the importance of psychophysics is once again beginning to be recognized.

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 the actual physical space (Figure 2). The discoverer Dr. Nijhawan has proposed that the human visual system uses the motion signals to extrapolate the position of a moving object (motion extrapolation hypothesis). 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 major two 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? In the present study, we are making an attempt to correctly interpret the flash-lag effect.

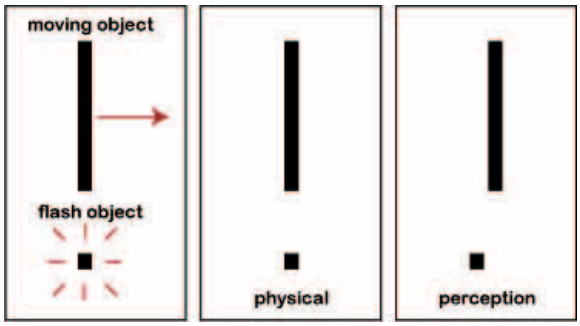

Figure 2. The flash-lag effect. A moving object is perceived to lead in space a flashed object when both objects are aligned in the actual physical space.

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. In addition, we have developed a novel method of conditional mutagenesis in mice in order to substitute the amino acid sequence of the target gene in particular cells. We analyze the physiological roles of the components of the dystrophin complex on the skeletal muscle membrane using genetically modified 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.

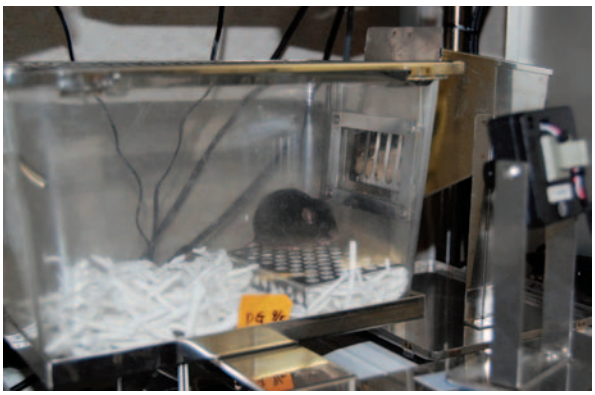

Figure 1. Experimental equipment for measurement of locomotor activity and food/water intake

In mammals, five subtypes of dopamine receptor (D1R, D2R, D3R, D4R and D5R) have been identified and divided into two subgroups, 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. D1R has an opposite property to D2R with respect to intracellular signal transduction.

In collaboration with Dr. Motoya Katsuki, Executive Director, National Institute of Natural Sciences, we have been investigating the involvement of dopaminergic transmission via D1R and D2R in the regulation of locomotion and eating behavior. We generated *D1R/D2R* double knockout (DKO) mice by crossing *D1R* knockout (KO) with *D2R* KO 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 molecular mechanism of motor control and eating behavior, we generated transgenic mice harboring tetracycline-regulated expression of the *D1R* gene on the *D1R/D2R DKO* background. Several transgenic mouse lines successfully rescued lethal phenotype of the *D1R/D2R* DKO mice and showed doxycycline (Dox) controllable expression of transgenic *D1R* gene (named as *D1R/D2R* DKO-*D1R* rescued mice). The *D1R/D2R* DKO-*D1R* rescued mice exhibited decreases in locomotion and food/water intake as well as a decrease in the amount of transgene expression after Dox administration. After the withdrawal of Dox administration, the *D1R/D2R* DKO-*D1R* rescued mice exhibited transient hyperactivity and recovered locomotor activity and food/water intake (Figure 2). We examined the expression levels of D1R protein in the striatum, which 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. We are analyzing these results to identify the mechanism of the relationship between the *D1R* expression and altered behavior. In addition, we are also investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

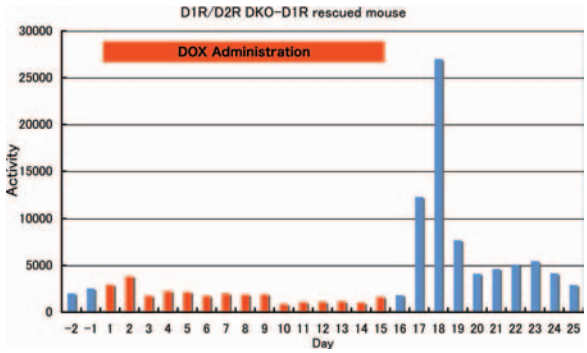

Figure 2. Locomotor activity of *D1R/D2R* DKO-*D1R* rescued mice. Two weeks of Dox administration led to decreased activity followed by transient hyperactivity.

Ⅱ. Developing a novel conditional mutagenesis method in mice

In collaboration with Prof. Yoichi Nabeshima of Kyoto University, we developed a novel mouse developmental biotechnology by introducing an amino acid substitution into a target gene in a spatially and temporally restricted manner.

The goal of the study was to overcome the limitations of the conventional mouse molecular genetic approach in the functional analysis of target genes. We substituted one critical amino acid residue (the 595th asparagines, Asp595) of N-methyl-D-aspartate receptor (NMDAR), leading to an aberrant activation of NMDAR. The NMDARs are widely expressed in the nervous system, fundamental to excitatory neurotransmission, and play a number of important roles at different brain loci and time points. The NMDARs act as a coincidence detector and are not only important for neuronal differentiation, migration, and survival, but are also critical for activity dependent synapse formation. It has been suggested that the aberrant activation of NMDAR causes excitotoxicity, leading to neuronal death of various neurological diseases.

Our method is vastly applicable to the functional analysis of any desired gene and should contribute to studies on the structural and functional relationships of relevant genes.

Ⅲ. Analysis of the roles of the sarcoglycan complex, dystroglycan complex and caveolin-3

Sarcoglycans (SGs) are trans-sarcolemmal glycoproteins that associate together to form sarcoglycan complex (SGC) and are present in the sarcolemma. SGC, together with dystrophin and the dystroglycan complex, comprises the dystrophin complex, which is considered to be the mechanical link between the basement membrane and the intracellular cytoskeleton for protecting the sarcolemma from mechanical stress during muscle contraction. Each of four SG subunits (α-, β-, γ- and δ-SG) is responsible for four respective forms of SG-deficient muscular dystrophy, sarcoglycanopathy (SGP). We generated the β-SG KO and γ-SG KO mice and found that the dystrophin complex isolated from the SG-deficient skeletal muscles was biochemically unstable. This indicates that SGC and sarcospan play an important role in stabilizing the dystrophin complex.

Caveolin-3 is a muscle-specific membrane protein, a component of the dystrophin complex, and serves as a scaffold for various molecules. Its gene mutations cause limb-girdle muscular dystrophy (LGMD1C or caveolinopathy) with mild clinical symptoms. In collaboration with Dr. Yasuko Hagiwara of Musashino University, Tokyo, we reported that *caveolin-3* deficiency causes muscle degeneration and a decrease in sarcolemmal caveolae in *caveolin-3* gene-knockout (*Cav3-/-*) mice. To examine the pathogenic pathways and identify new or modifying factors involved in caveolinopathy, we examined the gene expression profiles of approximately 8, 000 genes in the skeletal muscle of *Cav3-/-* mice using DNA microarray technique. We found that the gene of *osteoponin* (*OPN*), a versatile regulator of inflammation and tissue repair, was significantly down-regulated. This is in contrast to *mdx* mice showing a markedly up-regulated *OPN* gene in their skeletal muscles. Recently, *OPN* has been reported to be important in the pathogenesis of muscular dystrophy. We examined whether up-regulated *OPN* gene expression in *mdx* muscles is altered by the deficiency of caveolin-3. To this end, we developed caveolin-3 and dystrophin double-deficient mice. The levels of *OPN* mRNA and osteopontin in the double-deficient mice clearly decreased compared with those in *mdx* mice. We showed that although the level of *OPN* mRNA expressed in the double-deficient skeletal muscles was lower than that in *mdx* skeletal muscles, macrophage infiltration and muscle regeneration occurred similarly in the double-deficient and *mdx* skeletal muscles. There may still be other, undiscovered factors that are involved in macrophage infiltration and muscle regeneration.

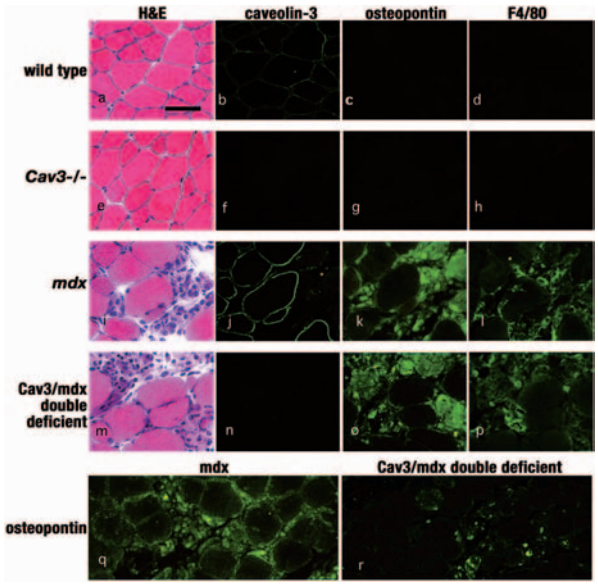

Figure 3. Immunohistochemical analysis of skeletal muscles from mice at 1 month of age. Cross sections were stained with H&E (a, e, i and m) and antibodies against caveolin-3 (b, f, j and n), osteopontin (c, g, k, o, q and r) and F4/80 (d, h, l and p). Note that the surrounding areas of F4/80-positive macrophages in *mdx* and double-deficient mice (l and p) are strongly stained by the anti-osteopontin antibody (k and o). In less infiltrated or noninfiltrated area, the sarcolemma of *mdx* muscle fibers is more strongly stained than that of the double-deficient muscle fibers by the anti-osteopontin antibody (q and r). Bar; 50 μm.

Publication List

〔Original papers〕

- Hagiwara, Y., Fujita, M., Imamura, M., Noguchi, S., and Sasaoka, T. (2006*). Caveolin-3 deficiency decreases the gene expression level of osteopontin in mdx mouse skeletal muscle. Acta Myol. *25*, 53-61.
- Kawasaki, K., Watabe, T., Sase, H., Hirashima, M., Koide, H., Morishita, Y., Yuki, K., Sasaoka, T., Suda, T., Katsuki, M., Miyazono, K., and Miyazawa, K. (2008). Ras signaling directs endothelial specification of VEGFR2+ vascular progenitor cells. J. Cell Biol. *181*, 131-141.

* a paper published after the publication of the previous issue of the Annual Report

DIVISION OF MOLECULAR GENETICS

Professor
IIDA, Shigeru

Assistant Professors	TERADA, Rie
	HOSHINO, Atsushi
	TSUGANE, Kazuo
Technical Staff	FUKADA-TANAKA, Sachiko
NIBB Research Fellow	MORITA, Yasumasa
Postdoctoral Fellows	JOHZUKA-HISATOMI, Yasuyo
	PARK, Kyeung-Il
	EUN, Chang-Ho
	MORITOH, Satoru
	TAKAGI, Kyoko
Graduate Student	SHIMATANI, Zenpei*
Visiting Scientist	YAMAUCHI, Takaki
Technical Assistants	ONO, Akemi
	TSUGANE-HAYASHI, Mika
	SHIMATANI, Zenpei
	SAITOH, Hiromi
	ASAO, Hisayo
	MATSUMOTO, Miwako
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	MATSUDA, Chisato
	HASEGAWA, Yoshinobu
Secretary	SANJO, Kazuko

The main interest of this division is understanding the biology of the dynamic genome, namely, genome organization and reorganization and its impact on gene expression and regulation. We are also characterizing various aspects of genetic and epigenetic gene regulations, particularly the flower pigmentation of morning glories. In addition, we are undertaking reverse genetic approaches in order to elucidate the nature of dynamic genome in rice, a model plant for cereals.

Ⅰ. Spontaneous mutants in morning glories

The genus *Ipomoea* includes about 600 species, distributed on a worldwide scale, that exhibit various flower morphologies and pigmentation patterns. Among them, three morning glories - *Ipomoea nil* (the Japanese morning glory), *Ipomoea purpurea* (the common morning glory), and *Ipomoea tricolor* - have been domesticated as floricultural plants. Of these, the Japanese morning glory has an extensive history of genetic studies, and about one-third of more than 200 spontaneous mutants described exhibit mainly altered flower pigmentation. The wild-type *I. nil* displays blue flowers with pigmented red stems and dark-brown seeds, and its spontaneous mutants with various flower colors have been isolated and cultivated in Japan since the 17th century. According to classical genetic studies, mutations conferring white flowers can be classified into four groups: *a*, *c*, *ca*, and *r*. The recessive *a* and *r* mutations confer white flowers with green stems and normal colored seeds. The first such mutation was originally designated *r*, and other recessive mutations that can complement the *r* mutation were named *a*. One of the *a* mutations was found to be caused by the integration of a DNA transposon *Tpn1* belonging to the CACTA superfamily into the *DFR-B* gene encoding dihydroflavonol 4-reductase. The *c* mutants exhibit white flowers with red stems and colored seeds, whereas the *ca* mutants display white flowers with green stems and ivory seeds. The *c* and *ca* mutations were identified as frameshift mutations in the genes encoding transcriptional regulators containing R2R3-MYB domains and those carrying conserved WD40 repeats (WDR), designated as InMYB1 and InWDR1, respectively. We found that the *r* mutants carry either 5.6-kb *Tpn3* or 4.7-kb *Tpn6*, DNA transposons of the *Tpn1* family, integrated into the *CHS-D* gene for chalcone synthase in the anthocyanin biosynthesis pathway (Figure 1). We designate these two mutant alleles as *r1-1* and *r1-2*. Because excision of *Tpn3* from *CHS-D* scarcely ever occurs, the *r1* mutant shows an apparent stable white flower phenotype. These findings further strengthen the assumption that the *Tpn1* family of elements act as major mutagens for generating various spontaneous mutations for floriculturally important traits in *I. nil*.

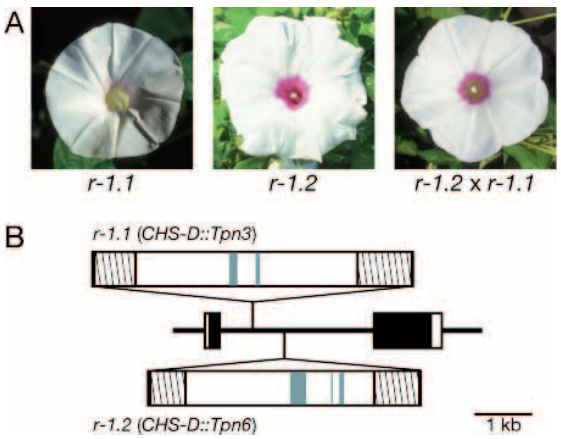

Figure 1. Flower phenotypes of the *r1* mutants and their *CHS-D* structures. (**A**) Flower phenotypes. (**B**) Mutant *CHS-D* genes. Boxes on the central horizontal bar indicate exons, with their coding regions shaded black. The transposons are shown above and under the horizontal bar.

Ⅱ. Modification of endogenous natural genes by homologous recombination in rice

Rice (*Oryza sativa*), with the sequenced genome of 389-Mb, is an important staple food for more than half of the world's population and is a model plant for other cereal species. Through a gene-targeting procedure with positive-negative selection, we have reported the generation of fertile transgenic rice plants with a positive marker inserted into the *Adh2* gene by using an *Agrobacterium*-mediated transformation vector containing the positive marker flanked by two 6-kb homologous segments for recombination. The vector used also contained the two constitutively expressed *DT-A* genes at both ends of the T-DNA segment adjacent to its border sequences, which acted effectively to eliminate transformants carrying entire T-DNA segment(s) integrated randomly and to enrich targeted transformants among the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

surviving calli. We found that base changes within the homologous segments in the vector could be efficiently transferred into the corresponding genomic sequences of rice recombinants. Interestingly, a few sequences from the host genome were flanked by the changed sequences derived from the vector in most of the recombinants (Figure 2). A likely explanation for the observation would be a result of the mismatch correction of heteroduplex molecules formed between the genomic and introduced T-DNA sequences during homologous recombination. These results offer new insights into the homologous recombination processes of gene targeting with positive-negative selection.

Because a single-stranded T-DNA molecule in *Agrobacterium*-mediated transformation is imported into the plant nucleus and becomes double-stranded, both single-stranded and double-stranded T-DNA intermediates can serve in gene-targeting processes. Since the *DT-A* gene is a cell-autonomous, nonconditional, and lethal negative selection marker, the *DT-A* gene region of a single-stranded T-DNA intermediate in the plant nucleus would neither become double stranded nor express transiently in the successfully targeted transformants because the transient expression of the double-stranded *DT-A* gene before integration is thought to kill host plant cells.

Several alternative models, including the occurrence of the mismatch correction of heteroduplex molecules formed between the genomic DNA and either a single-stranded or double-stranded T-DNA intermediate, are compared in order to explain the observation (Figure 3). Since the introduction of a double-strand break (DSB) in the plant genome greatly stimulates the integration of transgenes flanked by homologous sequences on T-DNA introduced into the DSB site, we considered (A) chromosomal DSB-initiated gene-targeting processes, and (B) chromosomal DSB-independent gene-targeting processes separately. According to the models illustrated, no heteroduplex would form in the

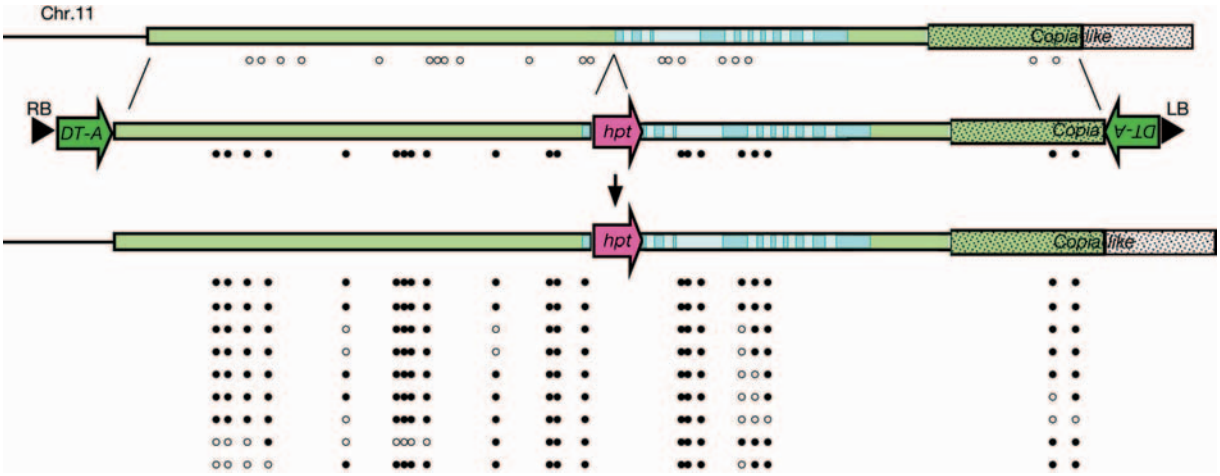

Figure 2. Transfer of base changes from a vector to the rice genome. The rice *Adh2* locus on chromosome 11 bears a *Copia*-like retroelement, and the vector used contains the 6.2-kb *Adh2* promoter sequence including a 0.1-kb 5'-untranslated region, *hpt*, 4.0-kb *Adh2* region, and 2.0-kb 3' part of a *Copia*-like retroelement. The large black arrowheads with RB and LB indicate the right and left borders of T-DNA, respectively. The blue boxes indicate *Adh2* exons, and *hpt* and *DT-A* are not drawn to scale. The filled and open circles indicate the base changes in the vector and the corresponding genomic sequences, respectively.

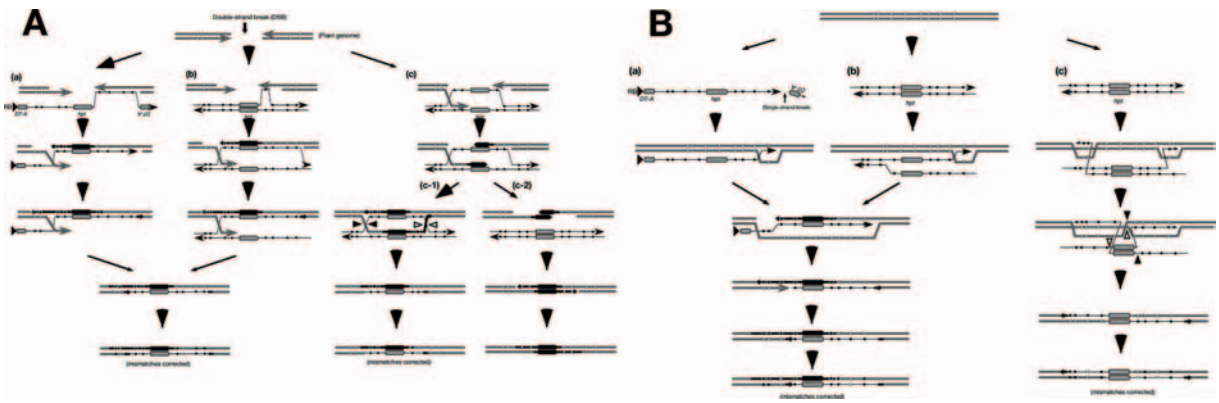

Figure 3. Models for gene targeting. (**A**) DSB-induced gene targeting processes with a single-stranded T-DNA intermediate (**a**) and a truncated double-stranded T-DNA intermediate (**b** and **c**). (**B**) DSB-independent gene targeting processes with a single-stranded T-DNA intermediate (**a**) and with a truncated double-stranded T-DNA intermediate (**b** and **c**).

simple DSB-induced synthesis-dependent strand annealing (SDSA) model involved in the annealing of the newly synthesized DNA strands (Figure 3A c-2), whereas a long heteroduplex would likely be produced in only one of the two homologous regions flanking the *hpt* gene in the formal double-strand break repair (DSBR) model. As the gene conversion was observed over the entire region examined, the observation appears to be in contrast to the fact that major DSB-induced conversion tracts were reported to be bidirectional and usually shorter than 100 - 300 bp when double-stranded DNA molecules served in homologous recombination with the genomic homologous sequences in mouse ES cells. We are condidering the possibility that a single-stranded T-DNA intermediate plays an important role in the major pathways leading to our successful GT events.

Ⅲ. Characterization of mutable *virescent* allele in rice

We have identified an active nonautonomous rice transposon *nDart1* of about 0.6 kb, belonging to the *hAT* superfamily, as a causative transposon of a mutable *virescent* allele *pyl-v* (*pyl-variegated*) conferring pale yellow leaves with dark green sectors in its seedlings. The transposition of *nDart1* can be controlled under natural growth conditions, and can be induced by crossing with a line containing an active autonomous element *aDart* and stabilized by segregating *aDart*. In the sequenced Nipponbare genome containing no active *aDart* elements, the *nDart*-related elements can be classified into three subgroups of about 0.6-kb nonautonomous elements and four subgroups of elements longer than 2 kb, which comprise epigenetically silenced inactive *iDart* and genetically defective *dDart* elements including 53 copies of *iDart1/dDart1* elements on the basis of their lengths and sequence characteristics. Among the 53 *iDart1/dDart1* elements, 38 *iDart1* elements are putative autonomous elements silenced epigenetically because their putative transposase genes carry no apparent nonsense or frameshift mutations. Because the excision of a fraction of *nDart1* elements can be induced by treating Nipponbare with 5-azacytidine (5-azaC), Nipponbare must contain an epigenetically silenced autonomous element or elements that can become *aDart* by 5-azaC treatment.

Mapping data indicated that *aDart* in the mutable pyl-v plants resides within 170-kb region on chromosome 6, and the 170-kb region contains a *Dart1* element identical to *Dart1-27* in Nipponbare. We chose six representative *Dart1* elements (*Dart1-1*, *Dart1-20*, *Dart1-27*, *Dart1-28*, *Dart1-44*, and *Dart1-52*) from Nipponbare to examine whether they are able to excise from the *GUS* gene in the vectors to be introduced into Arabidopsis by *Agrobacterium*-mediated transformation (Figure 4). Of these *Dart1* elements, only *Dart1-44* is likely to be *dDart1*, because its putative transposase gene contains a premature TAA stop codon. Transposition of all of the *Dart1* elements except for *Dart1-44* can be detected and expected footprints are generated, indicating that they are potential autonomous elements silenced epigenetically in Nipponbare and that autonomous *Dart1* elements bear a considerable sequence divergence of transposases. We also showed that *Dart1-27* can mobilize *nDart1-0* in Arabidopsis. 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.

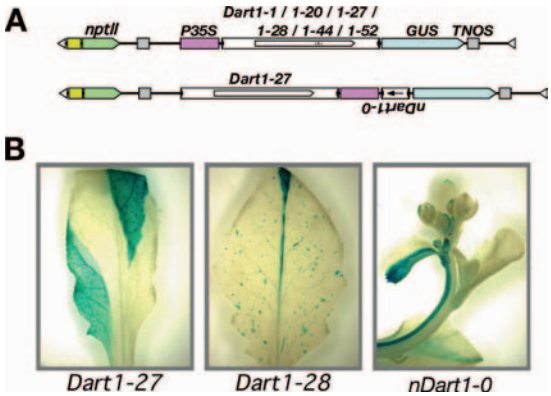

Figure 4. Transposision of *nDart1-0* and *Dart1* elements in Arabidopsis. (**A**) Structures of the T-DNA regions of the vectors used. (**B**) Excision activities of the introduced *nDart1-0* and *Dart1* elements examined in transgenic Arabidopsis plants.

Publication List

〔Original papers〕

- Johzuka-Hisatomi, Y., Terada, R., and Iida, S. (2008). Efficient transfer of base changes from a vector to the rice genome by homologous recombination: involvement of heteroduplex formation and mismatch correction. Nucleic Acids Res. *36*, 4727-4735.
- Kitazawa, D., Miyazawa, Y., Fujii, N., Hoshino, A., Iida, S., Nitasaka, E., and Takahashi, H. (2008). The gravity-regulated growth of axillary buds is mediated by a mechanism different from decapitation-induced release. Plant Cell Physiol. *49*, 891-900.
- Yamauchi, T., Moritoh, S., Johzuka-Hisatomi, Y., Ono, A., Terada, R., Nakamura, I., and Iida, S. (2008). Alternative splicing of the rice *OsMET1* genes encoding maintenance DNA methyltransferase. J. Plant Physiol. *165*, 1774-1782.
- Nishimura, H., Ahmed, N., Tsugane, K., Iida, S., and Maekawa, M. (2008). Distribution and mapping of an active autonomous *aDart* element responsible for mobilizing nonautonomous *nDart1* transposons in cultivated rice varieties. Theor. Appl. Genet. *116*, 395-405.

〔Review article〕

- Johzuka-Hisatomi, Y., Maekawa, M., Takagi, K., Eun, C.H., Yamauchi, T., Shimatani, Z., Ahmed, N., Urawa, H., Tsugane, K., Terada R., and S. Iida (2008). Homologous recombination-dependent gene targeting and an active DNA transposon *nDart*-promoted gene tagging for rice functional genomics. Biotechnology in Agriculture and Forestry *62*, 81-94

DIVISION OF GENOME DYNAMICS

Professor
HORIUCHI, Takashi

Assistant Professors	JOHZUKA, Katsuki
	WATANABE, Taka-aki
Technical Staff	MOROOKA, Naoki
Postdoctoral Fellow	KODAMA, Ken-ichi
Graduate Students	ITAZU, Masako
	OKAMOTO, Haruko
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 them and controlling instability are fully understood. To clarify these aspects, we are pursuing several lines of research using *Escherichia coli*, *Saccharomyces cerevisiae* and Chinese Hamster Ovary (CHO) cells. In 2008 we discovered a new role of rDNA in yeast and reported a relationship between condensin associating with the chromosome and gene transcription. More specifically, we discovered a new condensin recruiting system, consisting of the following four proteins, Tof2p, Csm1p, Lrs4p and Fob1p, which recruit condensin protein complexes to the *RFB* site in rDNA repeat units. In addition, we also developed a new system of gene amplification via DRCR (double rolling circle replication) in yeast using Cre-*lox* site-specific recombination. From previous and present results, we concluded that DRCR is an amplification mechanism actually used at least in budding yeast. We are investigating the possibility that DRCR might also act in gene amplification in higher eukaryotes as well.

Ⅰ. Analysis of mechanisms maintaining repeated structures of ribosomal RNA genes

In most eukaryotic organisms, the rDNAs are clustered in long tandem repeats on one or a few chromosomes. Although the total number of these rDNA repeats appears to be maintained at a level appropriate for each organism, genes with such a repeated structure are in general thought to be unstable because of a high frequency of recombinational events. Thus, it might be expected that organisms must have developed systems to regulate recombination within rDNA repeats.

In the yeast *Saccharomyces cerevisiae*, 200 copies on average are tandemly arrayed in a central position on the longest chromosome (XII). Recombinational events within the rDNA repeats in normal growing yeast cells appear to be mostly mediated by a *FOB1*-dependent system. *FOB1* is the gene required for replication fork blocking activity at replication fork barrier (*RFB*) sites, for rDNA region-specific recombination and for expansion/contraction of rDNA repeats. The latter two activities are likely to be triggered by double-strand breaks at the *RFB* site and repair of the break via gene conversion. Thus, *FOB1*-dependent recombination apparently contributes to the maintenance of average copy number of rDNA. However, in *Δfob1* cells, the repeats are still maintained without any fluctuation of copy number, suggesting that another, hitherto unknown, system acts to prevent contraction of the number of repeats.

In order to understand this putative alternative system, we collected a number of mutants in which the rDNA copy number decreased drastically under *Δfob1* conditions. We have found that mutations of genes encoding condensin caused this phenotype, suggesting that, in addition to its role in condensation and separation of chromosomes in M phase, condensin influences the maintenance of repeated rDNA structures. Each gene encoding a condensin subunit is known to be essential for growth, but the mutants isolated here are of leaky type. Analyzing the condensin and *fob1* double mutants and examining specific interactions between condensin and rDNA regions revealed that (1) in the double mutants, the copy number of rDNA in the mutant dramatically decreased; (2) the condensin complex associated with the *RFB* region in a *FOB1*-dependent manner; (3) the association between condensin and *RFB* was established during S phase and was maintained until anaphase; (4) double mutants showed slow growth which may be caused by a defect in the separation of the long rDNA array in anaphase. These results strongly suggest that *FOB1*-dependent condensin association with the *RFB* region is required for efficient segregation of rDNA repeat regions.

Ⅱ. Mechanism of condensin recruitment onto *RFB* sites located within the tandem rDNA repeat in budding yeast.

The primary functions of mitotic chromosome condensation are to reduce the length of the chromosomes to prevent truncation during cell division and to ensure proper segregation of sister chromatids. 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. 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 had only been shown at the resolution of light microscopy. The sites where condensin acts on the chromatin and the molecular mechanisms of condensin recruitment thereto had largely remained elusive. As described above, we found that condensin localized to the *RFB* site in a Fob1-dependent manner during S-phase. 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. Towards understanding chromosome condensation at the level of molecular resolution, we have studied mechanisms of condensin localization at the *RFB* site. Recently, we discovered that condensin could 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 condensin onto chromatin.

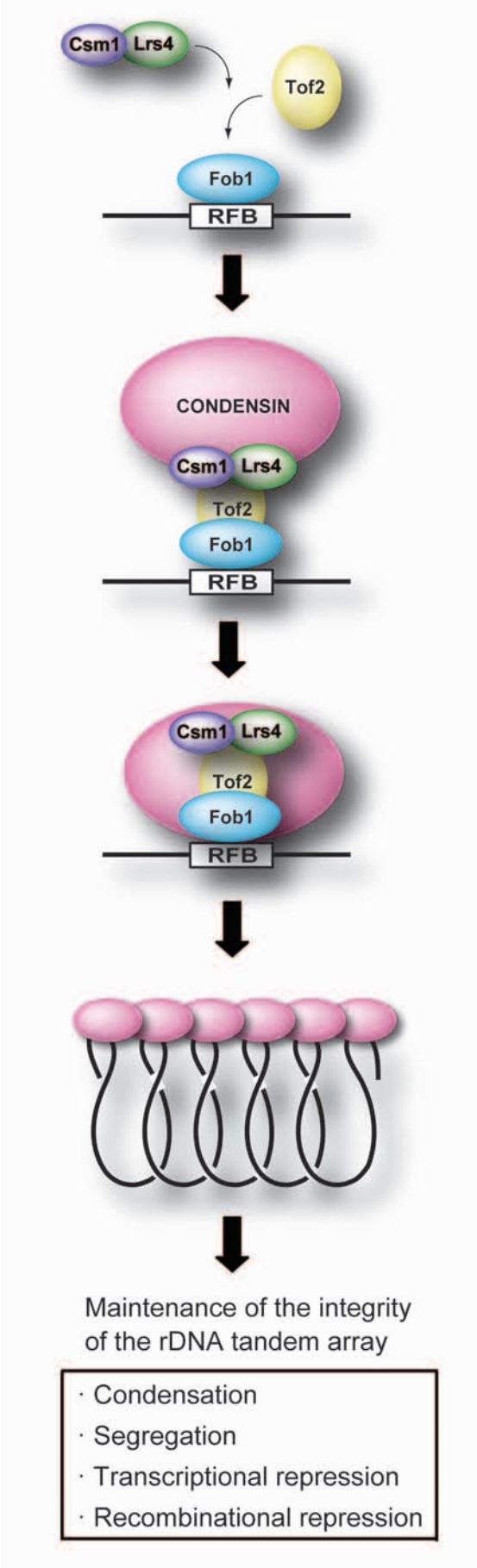

Figure 1. Model for condensin recruitment to the RFB site, contributing to maintenance of the integrity of long rDNA repeats.

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 complexes at the *RFB* site. Finally, *in vivo* interactions between Csm1p, Lrs4P and multiple subunits of condensin were detected. These results suggest that condensin is recruited to the *RFB* site by the sequential interactions of Fob1p, Tof2p, Csm1p, Lrs4p, and finally condensin, to ensure the proper segregation of long rDNA tandem arrays (Figure 1). (Johzuka, K. and Horiuchi, T. The *cis*-element and factors required for condensin recruitment to chromosome. *Mol. Cell*, in press)

Ⅲ. Gene amplification can be induced via excessive silencing caused by over-expression of Sir2p and Sir3p in *Saccharomyces cerevisiae*

Gene amplification is involved in various biological phenomena such as cancer development and drug resistance. Furthermore, amplification can be the first step in the evolution of a new gene because the extra copies are free to acquire new functions. However, the nature of the events causing gene amplification is poorly understood. We focused on gene silencing, an epigenetic mechanism for controlling of gene expression, and examined whether gene silencing affects gene amplification. A construct with the potential to induce gene amplification was inserted near a telomeric region in *Saccharomyces cerevisiae*, at which gene silencing was enhanced by *SIR2* or *SIR3* overexpression. This construct contains two markers, *URA3* and *leu2d*. The enhanced gene silencing is expected to suppress *URA3* expression and to lead to copy number increase that confers a growth advantage on organisms maintained in medium lacking uracil. We used the amplification marker, *leu2d* to detect the amplification of *URA3* with great sensitivity. The *leu2d* gene has only a slight transcriptional activity and complements leucine auxotrophy only when amplified. *SIR3* overexpression using the plasmid (YRp-*SIR3*) gave 700-fold higher frequency of Leu^+ survivors than the control. About 50% Leu^+ clones having a construct with inverted repeats contained intra-chromosomal products (>100 copies), and 16% Leu^+ clones contained extra chromosomal products (~20 copies). The remaining 32% of Leu+ clones contained 2-3 copies of the inserted structure. On the other hand, *SIR2* overexpression could cause gene duplication, although at low frequency. In addition, a type of chromosomal translocation was observed through introduction of an extra copy of *SIR2* (total 2 copies per cell) into cells harboring a construct with deliberate DNA cleavage. These results suggest that excessive levels of gene silencing might generate selective pressure and promote amplification of genes.

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

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, *leu2d* (up to ~730 kb increase) with novel arrangements present as randomly-oriented sequences flanked by inverted *leu2d* copies. Type-2 products are acentric multi-copy mini-chromosomes, each carrying two *leu2d* copies. Structures of type-1 and -2 products resemble those of homogeneously staining regions (HSR) and double minutes (DMs) of higher eukaryotes, respectively. Interestingly, products analogous to these were generated at low frequency without deliberate DNA cleavage (*EMBO J* 24, 190-198 (2005)).

If DRCR were an actual gene amplification mechanism in yeast, a quite different initiation reaction, which can induce DRCR, should produce amplification products resembling HSR and DMs. Thus, we tried to construct a new DRCR amplification system that is induced by another process, Cre-*lox* site-specific recombination. We first predicted that, if recombination occurs between un-replicated and replicated regions during replication, the fork will replicate these already-replicated regions again, and that the Cre recombination system would make this process more efficient, as shown in Figure 2(a). Furthermore, a combination of the processes, as shown in Figure 2(b), could efficiently induce gene amplification through DRCR. In fact, this system produced two kinds of products: highly amplified (>100 copies) chromosome products and acentric multi-copy extra-chromosomal products. Structures of these products resemble those of HSR and DMs of higher eukaryotes, respectively. From previous and present results, we concluded that DRCR is indeed an amplification mechanism in budding yeast and could be naturally initiated in the presence of the structural reguirement.

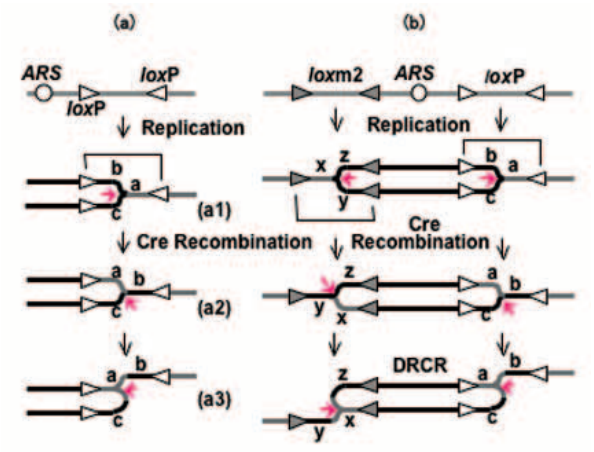

Figure 2. DRCR induced by the Cre-*lox* system
(a) Cre-*lox*-dependent reversal of replication orientation. (a1) When the replication fork passes between a pair of *lox* sites, Cre recombination occurs between them, as shown in (a1). Recombination changes replication orientation from un-replicated DNA (parental DNA strand) to replicated DNA (one of the sister chromatids) as shown in (a2 and a3), because DNA strand a and b are identical. (b) DRCR is induced by Cre-dependent combinational recombination of two pairs of *lox*P and *lox*m2 as shown in (b). Recombination cannot occur between *lox*P and *lox*m2.

DIVISION OF EVOLUTIONARY BIOLOGY

Professor
HASEBE, Mitsuyasu

Associate Professor
MURATA, Takashi

Assistant Professors	HIWATASHI, Yuji
	TANAHASHI, Takako
Technical Staff	SUMIKAWA, Naomi
NIBB Research Fellows	ISHIKAWA, Takaaki*
	MIYAZAKI, Saori
Postdoctoral Fellows	MIYAZAKI, Saori*
	ISHIKAWA, Takaaki
	MANO, Hiroaki
	OKANO, Yosuke
	OHSHIMA, Issei
Graduate Students	AOYAMA, Tsuyoshi
	FUJII, Tomomi
Visiting Scientist:	BASKIN, Tobias
Technical Assistants	IKEGUCHI, Hiromi
	ITO, Yukiko
	KAJIKAWA, Ikumi
	SAKURAI-OZATO, Nami
	TSUZUKI, Yukiko
	WASHIO, Midori
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 meristematic cell and a differentiated non-meristematic 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 plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards a cell periphery, and finally fuses to a parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. A 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.

In addition to the centrifugal expansion, antiparallel bundles in phragmoplasts are mostly unexplored and potentially offer new cellular insights. We found that the *Physcomitrella patens* kinesins KINID1a and KINID1b (for kinesin for interdigitated microtubules 1a and 1b), which are specific to land plants and orthologous to *Arabidopsis thaliana* PAKRP2, are novel factors indispensable for the generation of interdigitated antiparallel microtubules in the phragmoplasts of the moss *P. patens*. KINID1a and KINID1b are predominantly localized to the putative interdigitated parts of antiparallel microtubules. This interdigitation disappeared in double-deletion mutants of both genes, indicating that both KINID1a and 1b are indispensable for interdigitation of the antiparallel microtubule array. Furthermore, cell plates formed by these phragmoplasts did not reach the plasma membrane in approximately 20% of the mutant cells examined. We observed that in the double-deletion mutant lines,

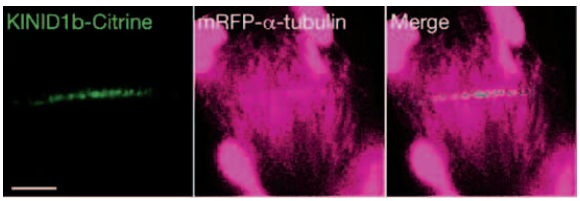

Figure 1. Localization of KINID1b-Citrine and mRFP-α-tubulin fusion proteins. The fluorescence derived from the mRFP-α-tubulin was of higher intensity at the phragmoplast equator where the plus ends of the antiparallel microtubules interdigitate. Fluorescent signals of KINID1b-Citrine protein overlapped with the high intensity mRFP signals. A bar = 2 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

chloroplasts remained between the plasma membrane and the expanding margins of the cell plate, while chloroplasts were absent from the margins of the cell plates in the wild type. This suggests that the kinesins, the antiparallel microtubule bundles with interdigitation, or both are necessary for the proper progression of cell wall expansion.

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 properly regulated during the development of multicellular organisms. Molecular mechanisms for the stem cell characterization, however, have been largely unknown. We showed that *AINTEGUMENTA/PLETHORA/BABY BOOM* (*APB*) orthologs *PpAPBs* (*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 *PpAPBs* play a critical role in the characterization of a gametophore stem cell. Meanwhile, the expression of *PpAPBs* is regulated by auxin, not cytokinin. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

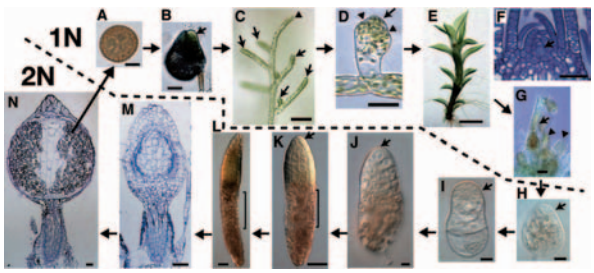

Figure 2. Life cycle of the moss *Physcomitrella patens*. Arrows indicate stem cells in different organs (see Sakakibara et al. 2008 in detail.)

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 was not possible because of the lack of genome sequences in basal land plants. We established an international consortium for a genome project of the basal land plant; subsequently, the moss *Physcomitrella patens* and its entire genome has been mostly sequenced as a collaborative work with the international consortium (Rensing et al. 2008).

To further elaborate the contig assembling and the gene annotation, we performed (1) the EST analyses of several libraries of cDNAs isolated from different developmental stages; (2) the construction of full-length cDNA libraries and sequencing in their full length; (3) the 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 available 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*

Polycomb group (PcG) proteins regulate chromatin modification and function as a cellular memory system to maintain the repressed state of developmental genes in both animals and plants. PcG genes are involved in phase changes of *Arabidopsis thaliana* development, such as vegetative to reproductive and haploid to diploid transitions. Bryophytes have dominant haploid generation, while sporophyte generation is dominant in angiosperms. The change of dominant generations is one of the most conspicuous evolutionary aspects of land plants. To elucidate the molecular mechanisms underlying the evolution in alteration of generations, the characterization of PcG genes in *P. patens* is in progress. Takaaki Ishikawa and Yosuke Okano were this study's primary researchers.

Ⅳ. Molecular mechanisms of female and male interactions

Sexual isolation is an important step for speciation, although the molecular mechanisms governing the isolation in plants are mostly unknown. A proper pollen tube guidance is essential for reproduction in angiosperms, and sexual isolation is often related to the arrest of guidance. In spite of the long history of studies on the pollen tube guidance, few guidance factors have been reported because of the difficulty of genetic analyses. We focused on receptors like kinases (RLKs), which function to receive extra cellular ligands and transmit the signal into a cell. We postulated that RLKs involved in pollen tube guidance are likely expressed more abundantly in pollen and/or pollen tube than in other tissue. Gene expression profiles between A. *thaliana* pollen and pollen tube were compared with those of other tissues using microarray. Pollen and pollen tube expression profiles were similar to each other and 95 % of expressed genes were overlapped within the 4 fold differences. We focused on 45 RLKs predominantly expressed in pollen or germinating pollen to characterize signaling mechanisms during fertilization. Characterization of single and double T-DNA insertion lines are in progress. This work was mainly done by Saori Miyazaki.

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, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. We attempted to elucidate the evolutionary mechanism of this complex mimicry at the molecular level. We first focused on the orchid mantis's mechanism of body coloration. Mass spectrometric analysis suggested that dihydro-xanthommatin, a red pigment belonging to the ommochrome family, contributes to the pink body coloration of the mantis. We also isolated a cDNA encoding a key enzyme for biosynthetic pathway of ommochromes. Using the sequence of this gene, we are now trying to establish RNAi-mediated gene knock-down in the orchid mantis.

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 the 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 for 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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LABORATORY OF MORRHODIVERSITY

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 apop-totic 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 en-gulfed 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.

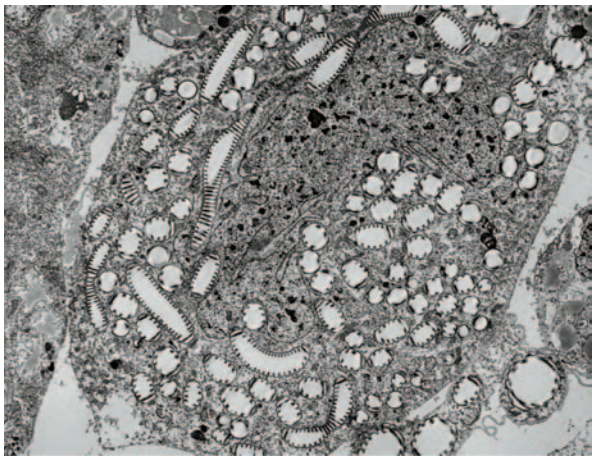

Figure 1. Transmission electron micrograph of the tracheole cell. Many cross-sections of the tracheoles can be observed within the cytoplasm of the tracheole cell. As the cell migrates, the tracheole is laid behind.

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. Our institute's Division of Sex Differentiation and the Laboratory of Neurophysiology were both involved in these activities.

LABORATORY OF BIORESOURCES

Associate Professor
NARUSE, Kiyoshi

Postdoctoral Fellows	SASADO, Takao
	TAKEHANA, Yusuke
	SHEN, Xueyan
Research Fellows	KANEKO, Hiroyo
	YOSHIMURA, Yuriko
Technical Assistants	KOIKE, Yukari
	TORII, Naoko
	AJIOKA, Rie
	KOIKE Chieko
Secretary	SUZUKI, Tokiko

Teleosts comprise about half of all vertebrate species and adapt 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. Construction of *Polypterus* Genomic DNA Library for Analysis of the Fish Genome Evolution

Comparative genomic analysis using medaka, zebrafish, *Tetraodon* and human as an outgroup revealed that the euteleost has experienced the teleost specific 3rd round genome duplication and the common ancestor of all euteleost should have 13 pre-duplicated proto-chromosomes. To verify this hypothesis, it is effective to compare the genomic structure of the reconstructed proto-chromosomes and fish chromosomes without the 3rd round genome duplication. One appropriate fish for this comparison is Polypteridae. We have started the construction of the genomic DNA library of *Polypterus senegalus* using fosmid vector and established the fosmid genomic library with 200,000 independent clones in 2008. In addition to this genomic resource, we are now establishing cell lines derived from the caudal fin of *Polypterus senegalus*.

Ⅱ. Evolution of the sex chromosome and sex determining 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

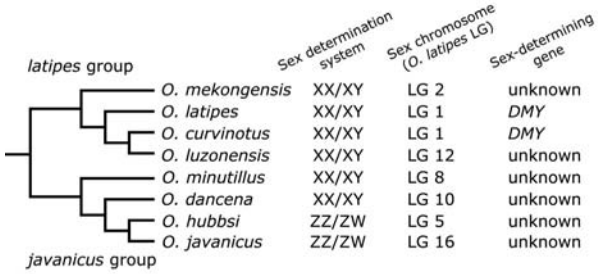

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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.

Ⅲ. Involvement of two chemokine receptors CXCR4b and CXCR7 in the regulation of the PGC migration

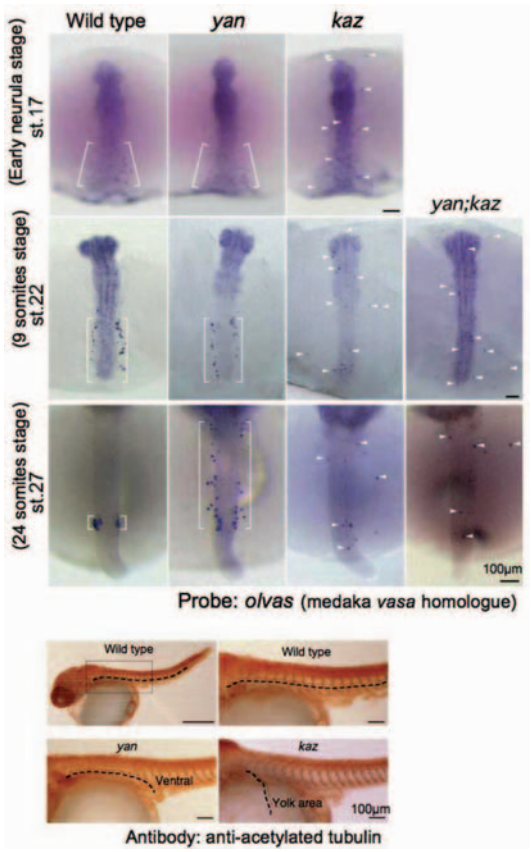

Figure 2. *yanagi* (*yan*) and *kazura* (*kaz*) mutations affect PGC migration and lateral line positioning.

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 teleost 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 (Figure 2). *kaz*$^{j2\text{-}15D}$ was identified as a missense mutation in chemokine receptor gene *cxcr4b* expressed in PGCs. Embryonic injection of *cxcr4b* mRNA with *olvas* 3'UTR rescued the PGC phenotype of *kaz* mutant, indicating a cell-autonomous function of *cxcr4b* in PGCs. *yan*$^{j6\text{-}29C}$ was identified as a nonsense mutation in the *cxcr7/rdc1* gene encoding another chemokine receptor. *cxcr7* transgene with genomic flanking sequences rescued the *yan* mutant phenotype efficiently at the G0 generation. *cxcr7* was expressed in somites rather than PGCs. *cxcr7*-expressing somitic domain expanded posteriorly with its margin immediately anterior of posteriorly drifting PGCs, as if PGCs were pushed toward the gonadal area. The *kaz* and *yan* mutants are also defective in the lateral line positioning, suggesting combined employment of these receptor systems in various cell migratory processes.

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

4-1 Full Length cDNA Sequencing Project

Although we published the draft genome sequence of medaka in 2007, the annotation of medaka genome is not yet well established. Sequencing of the full length cDNA clones is one of the most efficient methods for genome anotation. To do this, we made six full length cDNA libraries (developmental stage 22, 35, 40, ovary, brain and liver) and determined the sequences of both ends of 150, 000 clones in collaboration with the National Institute of Genetics' Kohara and Fujiyama labs. After mass alignment of all sequences, we found 16, 851 independent sequences. 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/).

4-2 Establishment of Core Facility of NBRP Medaka

In 2007, the 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 the integrated information on medaka (Figure 3). NBRP Medaka is aiming to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

Figure 3. NBRP Medaka website

Publication List

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- Ahsan, B., Kobayashi, D., Yamada, T., Kasahara, M., Sasaki, S., Saito, T. L., Nagayasu, Y., Doi, K., Nakatani, Y., Qu, W., et al. (2008). UTGB/medaka: genomic resource database for medaka biology. Nucleic Acids Res. *36*(Database issue), D747-D752.
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DIVISION OF MOLECULAR ENVIRONMENTAL ENDOCRINOLOGY

Professor
IGUCHI, Taisen

Associate Professor
WATANABE, Hajime

Assistant Professor	KATSU, Yoshinao
Technical Staff	MIZUTANI, Takeshi
NIBB Research Fellows	KATO, Yasuhiko
	KOIDE, Shizuyo
Postdoctoral Fellow	URUSHITANI, Hiroshi
Graduate Students	NAKAMURA, Takeshi
	KIKUCHI, Naoka
Visiting Scientists	TANIGUCHI, Ena
	MITSUI, Naoko
Technical Assistants	KOBAYASHI, Kaoru
	TANEMURA, Hiroyo
	HAYASHI, Tomoko
Secretary	IMAIZUMI, Taeko

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

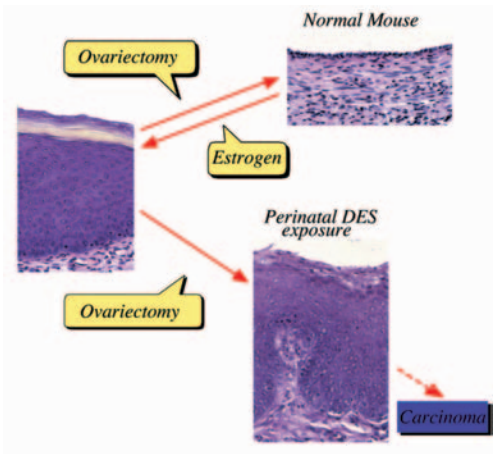

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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

The emerging paradigm of the "embryonic/fetal origins of adult disease" provides a powerful new framework for considering the effects of endocrine disrupters on human and animal health. In 1971, prenatal 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 phosphorylation of erbB2 and estrogen receptor α (ERα), sustained expression of EGF-like growth factors and phosphorylation of JNK1, IGF-I receptor and Akt. Recently, we found that Wnt 4 and other genes also show persistent expression changes in neonatally DES-exposed mouse vaginae. Currently, we are analyzing the methylation status of genes showing altered expression in the mouse vagina. The number of DES-induced genes in the mouse vagina during the critical developmental exposure window was smaller than after the critical period. We have demonstrated that neonatally exposed estrogen induced adverse effects mainly through ERα but not ERβ using specific ligands of ERs.

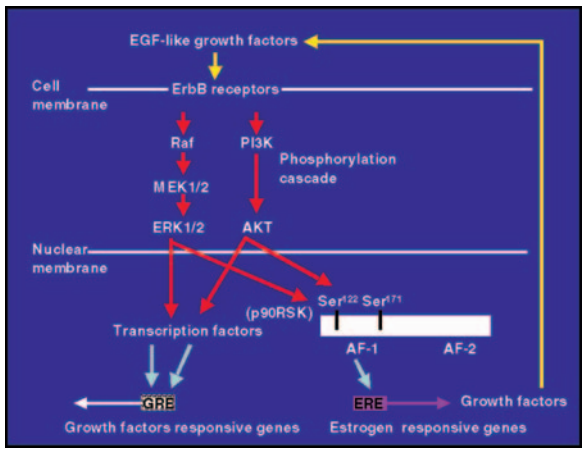

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

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 17 β-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 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.

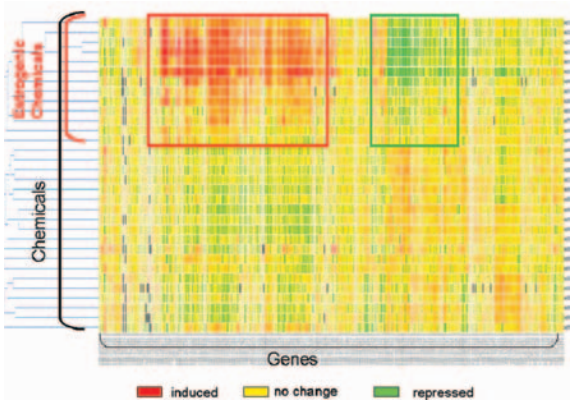

Figure 3. Scatter plot of average expression levels in control and chemical-treated uterus

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*, Japanese giant salamander, newt, *Silurana tropicalis*, American alligator, Nile crocodile, freshwater turtle, and 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.

Ⅳ. Male production by juvenile hormones in Daphnids

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

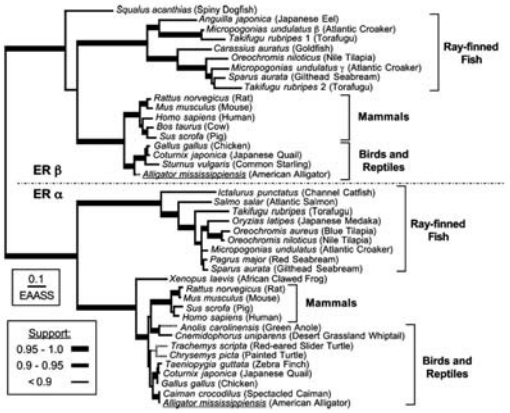

Figure 4. Evolutionary relationships of estrogen receptor sequences

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 these organisms. 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 silencing genes into *D. magna* embryos which will allow us to study gene function 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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DIVISION OF PLANT DEVELOPMENTAL GENETICS (ADJUNCT)

Professor (Adjunct)
TSUKAYA, Hirokazu

Assistant Professor	YAMAGUCHI, Takahiro
Postdoctoral Fellows	ISHIKAWA, Naoko
	USAMI, Takeshi
	YANO, Satoshi
Visiting Scientists	GOTOH, Ayako
	ICHIHASHI, Yasunori
	NAKAYAMA, Hokuto
	TAKASE, Masahide
Technical Assistants	YAMAGUCHI, Chinami
	NAGURA, Masako
	KADOWAKI, Tamaka
Secretary	KOJIMA, Yoko

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 2008.

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, however, we showed that AN does not have any of the molecular functions of CtBP in *Drosophila melanogaster* (Stern et al. 2007). If so, how widely is the AN function conserved in plants? We have isolated a homolog of AN from *Larix gmelinii*, a gymnosperm, and named it *LgAN*. *LgAN* fully complemented all known morphological phenotypes caused by *an-1* mutation in Arabidopsis, suggesting that the AN function is conserved between angiosperms and gymnosperms (Li et al. 2008). Furthermore, our detailed analysis of intracellular localization suggested that AN have a unique role (or roles) in Golgi-related functions. Further analyses of AN functions are ongoing.

On the other hand, constitutive over-expression of deletion series of ROT4 revealed that a 32-amino-acid core region is enough to exhibit the ROT4 function when over-expressed.

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 eudicot 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*. 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 the unifacial leaf formation; several interesting mutants of *Juncus* that exhibit abnormalities in leaf polarity were already isolated.

1-3 Size control of leaves and mechanisms of compensation

We have recently noticed that leaf organogenesis depends on "leaf meristem" that is seen only in the border region between leaf blade and leaf petiole. All cells required for leaf formation seem to be supplied from this leaf meristem. How are cell proliferation and cell enlargement coordinated in leaf morphogenesis? In a determinate organ - such as a leaf - the number of leaf cells is not necessarily reflected in leaf shape or, more particularly, 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 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 the number of leaf cells, *fugu* mutants that exhibit typical compensation syndrome, namely, decreased number of cells and increased cell volume, and *msc* mutants that exhibit an "opposite-type" compensation syndrome, namely an increased number of cells and decreased cell volumes.

This year we have revealed that: (1) *fugu5* phenotype is cancelled by supplying sucrose to the growth medium; (2) several *oli* mutations are loss-of-function mutations of ribosome biogenesis genes; (3) "opposite-type" compensation syndrome in *msc* mutants is attributed to accelerated heteroblasty (Usami et al., 2009); detailed analyses of this phenomenon strongly suggested that traits of heteroblasty are regulated by several different pathways. Furthermore, a new tool for studies of the mechanisms of compensation, chimeric expression system of *KRP2* or *AN3*, was established, and several candidate genes responsible for the compensation were selected from microarray analysis of *fugu2* and *an3*.

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 (Figure 1). These facts suggest that AN3 is involved in various key aspects of organogenesis in Arabidopsis. Further analyses of the mechanisms of compensation are in progress.

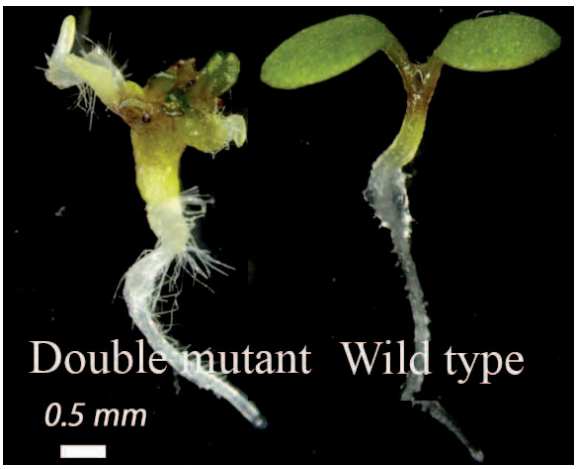

Figure 1. Ectopic root formation is seen on cotyledon region in *an3-#2047* double mutant. Bar, 0.5 mm.

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. Curiously, plants with high-ploidy syndrome have more than eight sets of homologous chromosomes (8C), resulting in an increase in cell volume, but have smaller leaves (Tsukaya 2008).

The construction of a series of tetraploids of leaf shape/size mutants supplied us with a good clue for understanding the linkage mechanisms between the ploidy level and cell/organ size. 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. This year we analyzed several achlorophyllous mycoheterotrophs. Mophological and molecular phylogenetic analyses revealed that *Monotropastrum humile* var. *glaberrimum* must not be conspecific to *M. humile* (Tsukaya et al. 2008). Molecular phylogenetic analysis of *Oxygyne shinzatoi* showed that this very rare genus would be basal taxon of tribe Thismieae (Yokoyama et al. 2008). Moreover, we have found a new species of the genus *Oxygyne*, *O. yamashitae* from Yakushima island (Yahara and Tsukaya 2008). This is the third species of this genus reported from Japan. Yakushima Island was proven once again to be a hot spot for plant biodiversity in Japan.

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〔Review article〕

- Tsukaya H. (2008). Controlling Size in Multicellular Organs: Focus on the Leaf. PLoS Biol. *6*, 1373-1376.

DIVISION OF PHOTOBIOLOGY (ADJUNCT)

Professor (Specially appointed)
WADA, Masamitsu

Associate Professor (Adjunct)
YAMAUCHI, Daisuke

Postdoctoral Fellows	KAGAWA, Takatoshi
	KODAMA,Yutaka
	KONG, Sam-Geun
	SUETSUGU, Noriyuki
	YAMASATO, Akihiro
Visiting Scientist	TSUBOI, Hidenori
Technical Assistants	CHUNG, Hee-Jung
	HIROSE, Mana
	IWATA, Ayumi
	SHIMIZU, Mineko
Secretary	TAKAMATSU, Kaori

Plants respond to light as an environmental factor to optimize growth and development and to regulate other physiological phenomena. Phytochrome (phy) and blue light receptors, such as cryptochrome (cry) and phototropin (phot), are the main photoreceptors for plant photomorphogenesis. The goal of our research is to elucidate the photoperception and signal transduction pathways of photomorphogenesis. One of our major subjects is chloroplast photo-relocation movement, which is mediated by phototropins and is one of the simplest model systems for studying photomorphogenesis. Because the phenomenon is cell autonomous, whole processes from photoperception to chloroplast movement can be accomplished in a single cell without any influence from surrounding neighbor cells. Moreover, gene expression is not involved in the signal transduction pathways, unlike in those of phy- and cry-mediated phenomena. Chloroplast movement is not real plant morphogenesis, but we are studying it because chloroplast movement and photomorphogenesis share the same photoreceptors.

Ⅰ. Chloroplast relocation movement

We use the fern *Adiantum capillus-veneris* as a model plant for our cell-biological and physiological approach to chloroplast movement since the gametophytes are very sensitive to light and the organization of the cells is very simple. We also use *Arabidopsis* mutants as well as wild type plants to identify the genes regulating chloroplast movement and for analyses of the genes' functions.

1-1 Polarity of moving chloroplasts

Chloroplast movement can be induced by whole cell or partial cell irradiation and is easily observed under a microscope. Since the speed of movement is very low, the detection of chloroplast movement during real time observation is not easy, so that chloroplast movement is usually recorded using time lapse movies. The figures obtained every minute were analyzed precisely.

When *A. capillus-veneris* gametophytes were incubated in darkness for two or three days, most chloroplasts moved to the anticlinal wall but only a few chloroplasts could be found on the periclinal wall. Chloroplast movement was induced by partical cell irradiation with sequential microbeams at different areas near the chloroplasts on the periclinal walls. Precise analyses of the chloroplast behavior revealed that chloroplasts can change direction without turning, although a time lag of a few minutes is required for

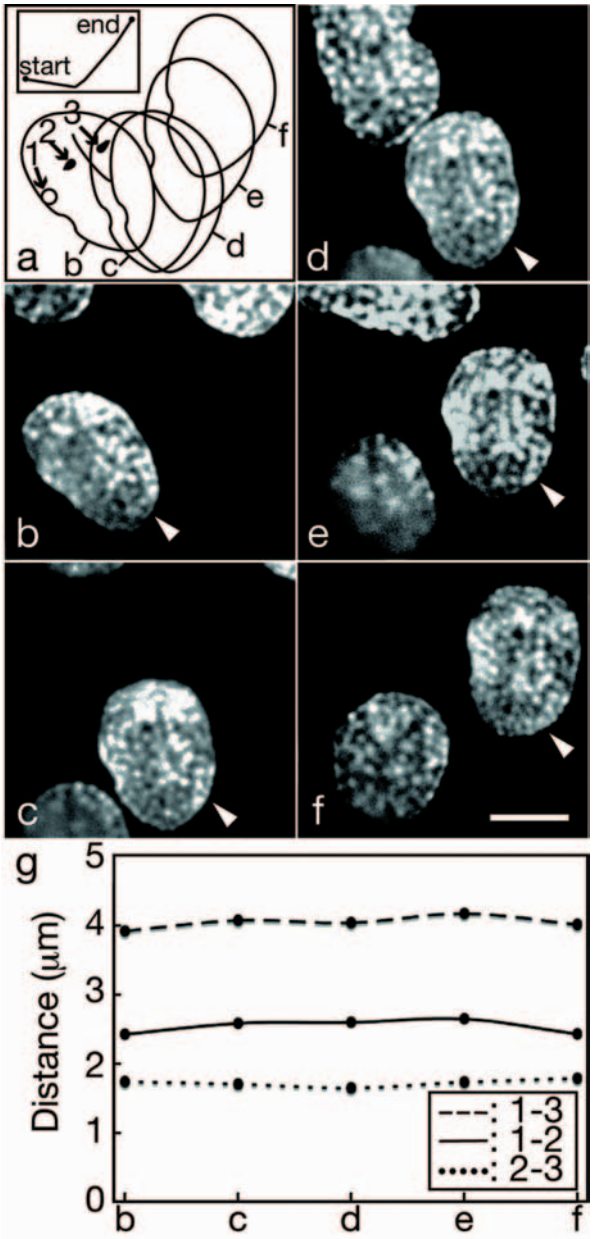

Figure 1. Dark-adapted A. *capillus-veneris* gametophyte was irradiated with red light (4.5 μmol m-2 s-1) to induce chloroplast movement from anticlinal wall to periclinal wall. The change in the distribution pattern of grana stucks during the movement was analyzed every 5 minutes. (a) Outlines of a chloroplast and the path of the center of the chloroplast (inset) during the movement. (b–f) Fluorescent micrographs of chloroplasts taken every 5 minutes. (g) The distances among grana stucks shown as 1, 2 and 3 were plotted (from Journal of Plant Research Tsuboi *et al*. 2009).

this. During the movement chloroplasts do not roll but slide, keeping the concave side to the plasma membrane. These chloroplast behaviors are the same under both blue and red light micorobeam irradiation.

1-2 Functional analysis of CHLOROPLAST UNUSUAL POSITIONING 1 protein in chloroplast movement

CHLOROPLAST UNUSUAL POSITIONING 1 (CHUP1) protein has a hydrophobic domain at its N-terminus and localizes on a chloroplast outer envelope. CHUP1 also has an F-actin binding domain and a proline rich region for profilin binding, suggesting the possible role of actin polymerization. The mutant deficient in CHUP1 protein (*chup1*) does not show chloroplast photo-relocation movement, so that chloroplasts sediment at the palisade cell bottom. The function of CHUP1 was studied using the transformants with variously truncated cDNA of *CHUP1* genes in *chup1* background (Oikawa et al 2008). Interestingly, when the CHUP1 N terminus hydrophobic region was replaced with the hydrophobic domain of a chloroplast outer envelope protein 7 (OEP7)-GFP, the chup1 phenotype was complemented, although both amino acid sequences are quite different. The results indicate that the amino acid sequence of CHUP1 N terminus is not important but that its hydrophobic character is essential for the CHUP1 function to attach the functional part of CHUP1 to the chloroplasts.

The coiled-coil region of CHUP1 anchors chloroplasts firmly on the plasma membrane, consistent with the localization of coiled-coil-GFP on the plasma membrane.

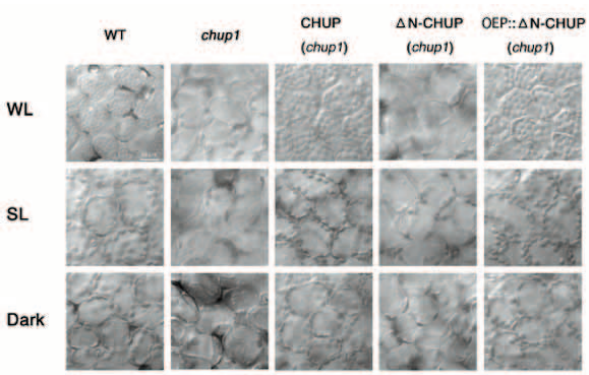

Figure 2. Distribution patterns of chloroplasts in each transgenic line under darkness (Dark), white light (WL), and strong white light (SL) conditions were compared among wild type, *chup1*mutant and the three transgenic plant lines (transferred with a full length *CHUP1* gene (CHUP), with *CHUP1* without N-terminus (ΔN-CHUP), and with *CHUP1* with which CHUP1 N terminus was replaced with a N terminus hydrophobic region of the outer envelope protein 7 (OEP::ΔN-CHUP)), CHUP1 whose N terminus was replaced with OEP7 N terminus rescued *chup1* mutant as *CHUP1* transgenic plants did. The experiments were repeated at least three times. Bar = 10 mm.

Publication List

〔Original papers〕

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〔Review articles〕

- Wada, M. (2008). Photoresponses in fern gametophytes. In: The Biology and Evolution of Ferns and Lycophytes" Edited by Tom A. Ranker and Christopher H. Haufler, Cambridge Univ. Press. Pp. 3-48.
- Ogura, Y., Tokutomi, S., Wada, M., and Kiyosue, T. (2008). PAS/LOV proteins: A proposed new class of plant blue light receptor. Plant Signaling & Behavior *3*, 966-968.

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 intrinsic effector role in *Euglena gracilis* (Figure 1), a unicellular flagellate alga, which shows blue-light type photomovements (Iseki *et al.*, Nature *415*, 1047-1051, 2002). 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, has been considered a photosensing organelle responsible for the 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.

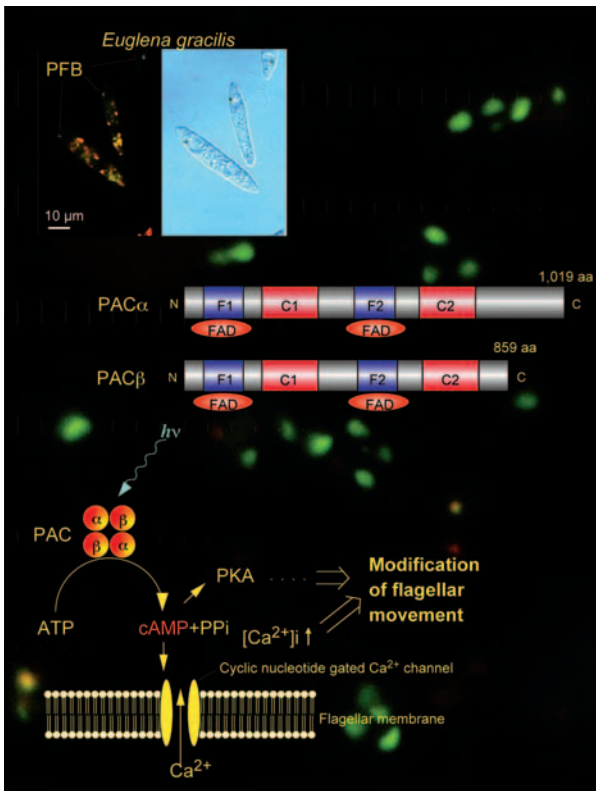


Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

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. Structures and features of the photoreceptive domains (F1 and F2) of PAC

To biophysically understand intramolecular photosignal processing, knowledge of the three dimensional structure of

the protein as well as spectroscopical analyses are essential. Due to the present difficulty of preparative heterologous expression of functional PAC, however, neither crystallography nor spectroscopical analyses of the whole PAC protein have been realized yet. To partially compensate for this situation we tried homology modeling and quantum chemical calculation of its photoreceptive domains (F1 and F2) of the α- subunit using their prokaryotic counterparts, BLUFs, as the templates (Figure 3). It was of interest that, from the viewpoint of binding energies thus calculated, F1 appeared to bind FAD less strongly than F2 does. This difference might indicate different structural and functional roles between these very similar domains.

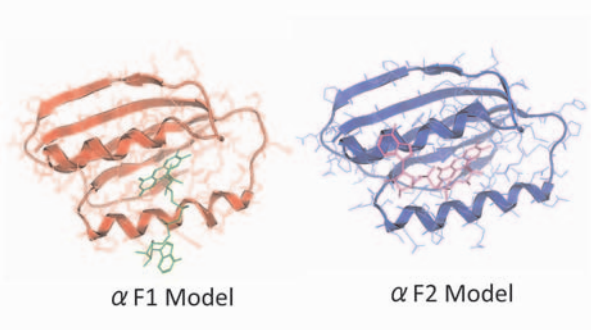


Figure 3. Three-dimentional structure model of the photoreceptor domains (F1 and F2) of PACa subunit. The template for the homology modeling is AppA, a bacterial blue-light sensor.

Publication List

〔Original papers〕

- Maruyama, S., Misawa, K., Iseki, M., Watanabe, M., and Nozaki, H. (2008). Origins of a cyanobacterial 6-phosphogluconate dehydrogenase in plastid-lacking eukaryotes. BMC Evol. Biol. *8,* 151 doi:10.1186/1471-2148-8-151.
- Ioki, M., Takahashi, S., Nakajima, N., Fujikura, K., Tamaoki, M., Saji, H., Kubo, A., Aono, M., Kanna, M., Ogawa, D., Fukazawa, J., Oda, Y., Yoshida, S., Watanabe, M., Hasezawa, S., and Kondo, N. (2008). An unidentified ultraviolet-B-specific photoreceptor mediates transcriptional activation of the cyclobutane pyrimidine dimer photolyase gene in plants. Planta *229*, 25-36.
- Mori, E., Takahashi, A., Kitagawa, K., Kakei, S., Tsujinaka, D., Unno, M., Nishikawa, S., Ohnishi, K., Hatoko, M., Murata, N., Watanabe, M., Furusawa, Y., and Ohnishi, T. (2008). Time course and spatial distribution of UV effects on human skin in organ culture. J. Radiat. Res. (Tokyo) *49*, 269-277.

DIVISION OF THEORETICAL BIOLOGY

Professor (Concurrent)
MOCHIZUKI, Atsushi

Associate Professor	MOCHIZUKI, Atsushi*
NIBB Research Fellow	FUJITA, Hironori
Postdoctoral Fellows	ISHIHARA, Shuji
	NAKAZATO, Kenichi
Graduate Student	IMAMURA, Hisako
Secretary	UMEBAYASHI, Hiromi

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. 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 (Figure 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 (Figure 2b). (C) Multiple loops that are connected by sharing the same genes do not increase the maximum diversity of steady states (Figure 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.

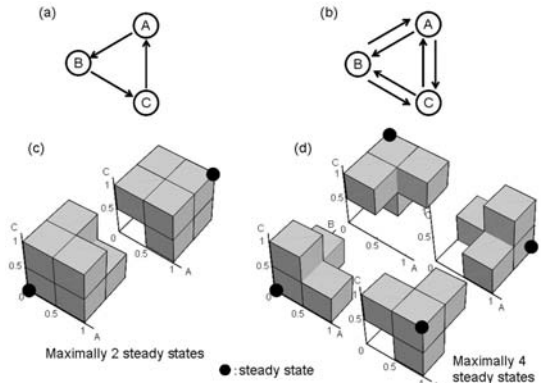


Figure 1. An intuitive explanation of "steady state compatibility". (a) An example of the regulatory links of a mono-directional loop with three genes. (b) Another example of the regulatory links of a bi-directional loop with three genes. (c) The shaded domains show the region where other steady states 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.

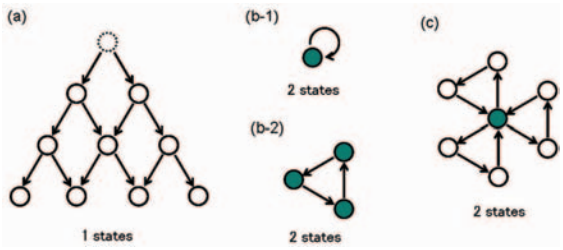


Figure 2. General properties showing the relationships between the structure of regulatory networks and the maximum diversity of steady states.

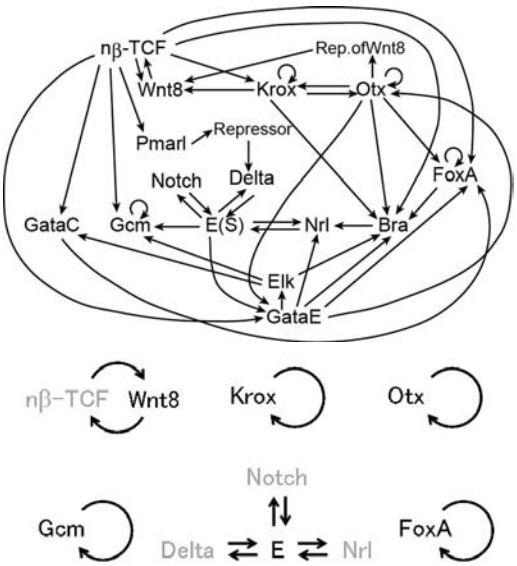


Figure 3. Analysis of an actual gene network responsible for the early development of a sea urchin species. (a) The network is simplified from the one of Fig. 3 in Davidson *et al*. (2002). The maximum diversity generated from this network is determined by the analysis as 64. (b) All of the "reduced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

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

Ⅱ. Predicting regulation of the phosphorylation cycle of KaiC clock protein using mathematical analysis

Cyanobacteria are the simplest organisms exhibiting circadian rhythms. In the bacterium, clock genes kaiA, kaiB and kaiC have been characterized as the indispensable clock regulators. KaiC plays a central role and exhibits rhythms in transcription, translation and phosphorylation status under continuous illumination conditions. The other clock proteins KaiA and KaiB modulate KaiC autophosphorylation: KaiA enhances autophosphorylation of KaiC, and KaiB inhibits this action of KaiA. It was recently revealed that periodic oscillation of the phosphorylation level of KaiC persists even under continuous dark conditions, where transcription and translation have almost ceased. The KaiC phosphorylation cycle was reconstituted even *in vitro*, thus confirming that the interaction between Kai proteins generates the cycle, although the specific mechanism that drives the clock remains unclear.

Using mathematical models, we investigated the mechanism for the transcription-less KaiC phosphorylation cycle. We developed a simple model based on possible KaiC behavior suggested by previous experimental studies. In the model, the KaiC-KaiA complex formation followed by a decrease in free KaiA molecules may attenuate the KaiC phosphorylation rate, and it may acts as negative feedback in the system. However, our mathematical analysis proved that simple dynamics based on the experimentally suggested model never show the KaiC phosphorylation cycle.

We then developed the generalized formulae of models and determined the necessary condition to generate the KaiC phosphorylation cycle. Linear stability analysis revealed that oscillations can occur when there is sufficient distance of feedback between the recipient reaction and the effector. Furthermore, we found that the negative feedback regulations in closed systems can be classified into two types: *destabilizing inhibition* and *stabilizing inhibition*.

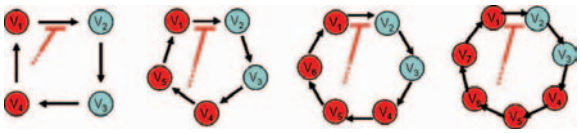


Figure 4. Schematic representation of closed circuit model and the condition for the possible oscillation by inhibition of the transition from state V_1 to V_2. Red-colored state (V_1, V_4-V_7) indicate that inhibition from the states can destabilize the system and possibly cause oscillation. Inhibition from the blue-colored state never induces oscillation.

# of states	inhibitor V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
3	O	×	×	-	-	-	-	-
4	O	×	×	O	-	-	-	-
5	O	×	×	O	O	-	-	-
6	O	×	×	O	O	O	-	-
7	O	×	×	O	O	O	O	-
8	O	×	×	O	O	O	O	O

×: always stable
O: oscillation possible

Table 1. Summary of the results of the general state transition model with conservation of molecules. The system could oscillate when the inhibiting state is more than two steps ahead of the inhibited reaction (from V_1 to V_2). If the inhibiting state is less than three steps ahead of the reaction, the system is always stable. The necessary distance between the inhibiting state and reactant state does not depend on the system size.

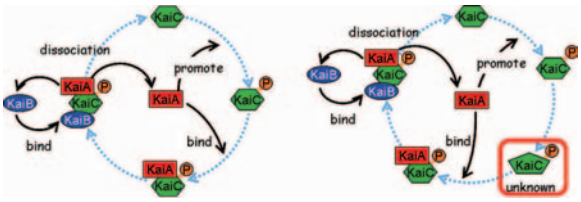


Figure 5. Schematic representations of "Basic model" (left) and "Multiple-phosphorelation-state model" (right). The basic model is determined from experimental results. It was proven that the model never shows oscillation. The multiple-phosphorelation-state model was developed based on a mathematical analysis. The model shows clear periodic oscillations. There are at least two different phosphorelated states. The time-delay caused by the transition between the states is essential for generating oscillation.

Based on this result, we predicted that, in addition to the identified states of KaiC, another unknown state must be present between KaiC phosphorylation and the complex formation. By incorporating the unknown state into the previous model, we realized the periodic pattern reminiscent of the KaiC phosphorylation cycle in computer simulation. This result implies that the KaiC-KaiA complex formation requires more than one step of posttranslational modification including phosphorylation or conformational change of KaiC. This prediction has recently been confirmed by experimental methods.

Publication List

〔Original paper〕

- Mochizuki, A. (2008). Structure of regulatory networks and diversity of gene expression patterns. J. theor. Biol. *250*, 307-321.

LABORATORY OF GENOME INFORMATICS

Assistant Professor
UCHIYAMA, Ikuo

The accumulation of biological data has recently been accelerated by various high-throughput "omics" technologies such as genomics, transcriptomics, proteomics, and so on. The field of genome informatics is aimed at utilizing this data, or finding some 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. Extracting useful information from such a growing number of genomes is a major challenge in genomics research. Interestingly, many of the completed genomic sequences are closely related to each other. We are developing methods and tools to conduct comparative analyses not only of distantly related genomes but also of closely related genomes, since we can extract different types of information about biological functions and evolutionary processes from comparisons of genomes at different evolutionary distances.

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 III below). By this algorithm, MBGD not only provides the 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. The constructed classification table can be used for comparative analyses from various points of view, such as phylogenetic pattern analysis, gene order comparison, and detailed gene structure comparison. For researchers who are interested in ongoing genome projects, MBGD also provides a service called "My MBGD," which allows users to add their own genome sequences to MBGD for the purpose of identifying orthologs among both the new and the existing genomes.

The database now contains around 700 published genomes including 13 eukaryotic microbes (fungi and protozoa) and *C. elegans* as a reference. MBGD is available at http://mbgd.genome.ad.jp/.

Ⅱ. 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, such as genomic islands. Therefore, 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. Here, 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 (OGs) that retains to the greatest possible extent the conserved gene orders.

The program, named CoreAligner, requires a set of well-conserved OGs. We compiled them using the MBGD server, and considered an OG "conserved" when it was present in at least half of the genomes. Next, a neighborhood graph was constructed using a set of conserved neighborhood pairs, which are defined as two conserved OGs that are located within 20 genes in at least half of the genomes. Our algorithm for constructing the alignments of the core genome structures is based on finding the longest path of the conserved neighborhood graph. A similar algorithm was previously developed mainly for identifying much shorter but more widely conserved gene clusters such as operons, but unlike that method, our method considers not only genes in the same direction but also those in the opposite direction as neighboring genes, and thereby generally generates longer alignments. In addition, our method uses the dynamic programming algorithm for calculating the longest path.

The method was applied to genome comparisons of two well-characterized families, *Bacillaceae* and *Enterobacteriaceae*, and identified their core structures comprising 1438 and 2125 OGs, respectively (Figure 1), which correspond to a third of the number of the B. *subtilis* genes (4105) and half of the E. *coli* genes (4237), respectively. The core sets contained most of the essential genes (90%) and their related genes, which were primarily included in the intersection of the two core sets comprising around 700 OGs. The definition of the genomic core based on gene order conservation was demonstrated to be more robust than the simpler approach based only on gene conservation. We also investigated the core structures in terms of G+C content homogeneity and phylogenetic congruence, and found that the core genes primarily exhibited the expected characteristic (i.e., being indigenous and sharing the same history) more than the non-core genes.

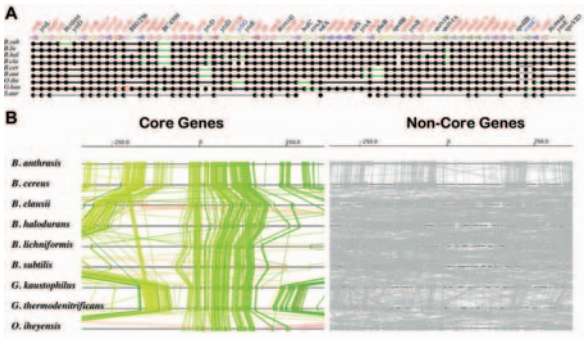


Figure 1. Core genome alignment. (A) Part of a schematic representation of genome alignment obtained from the *Bacillaceae* dataset. (B) Comparative genome map showing the locations of the core (left) and non-core (right) genes, where the latter are extensively crossed with each other.

Ⅲ. 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.

As the number of completed genomic sequences grows, comparison among closely related as well as distantly related genomes has become important for understanding the function and evolution of various genomes. For evolutionary analysis of a target set of related organisms (ingroup), we often need another set of organisms outside of that group (outgroup) to correctly infer evolutionary processes. To incorporate this concept into the ortholog analysis, we have enhanced the DomClust algorithm to impose constraints on the resulting orthologous groups so that the outgroup species should come outside of the ingroup species. The resulting table has a nested structure when a duplication event occurs within the ingroup lineage.

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 the above-described approaches including the ingroup/outgroup distinction in ortholog grouping and the core structure extraction among related genomes. In addition, the RECOG system has several advanced features that allow users to perform more flexible phylogenetic pattern analyses.

The entire RECOG system employs client-server architecture. The RECOG server program has been developed based on the MBGD server and contains database construction protocol in MBGD including all-against-all similarity search calculation, as well as the extended version of the DomClust program. Unlike MBGD, however, users can install the RECOG server on their local machines to analyze their own genomic data. Alternatively, users can connect to the public RECOG server to analyze available data.

The RECOG client program is a Java application that runs on a local machine by receiving data from any available RECOG server. The main window of the RECOG client consists of three parts: taxonomic tree viewer (left), ortholog table/phylogenetic pattern map viewer (center) and gene information viewer (right). Users can choose a set of genomes to compare in the taxonomic tree viewer and run the DomClust program to identify orthologous groups among them. The result is displayed in the ortholg 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. Several sorting and filtering functions have been implemented for modifying the display of the ortholog table viewer, including sorting by functional category or by the gene order of a specified genome, and filtering by keywords or by phylogenetic pattern conditions. The RECOG system also contains an interface for the CoreAligner program, by which users can see an alignment display and a comparative map display of conserved core regions identified by the CoreAligner program (Figure 2).

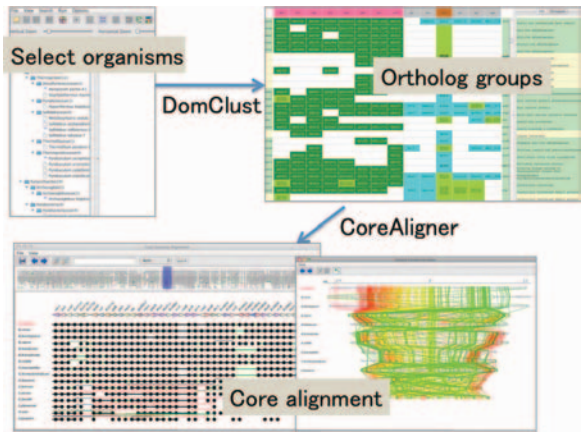


Figure 2. A typical usage of the RECOG system including DomClust and CoreAligner analyses.

Publication List

〔Original papers〕

- Uchiyama, I. (2008). Multiple genome alignment for identifying the core structure among moderately related microbial genomes. BMC Genomics, *9*, 515.
- Nakayama, K., Yamashita, A., Kurokawa, K., Morimoto, T., Ogawa, M., Fukuhara, M., Urakami, H., Ohnishi, M., Uchiyama, I., Ogura, Y., Ooka, T., Oshima, K., Tamura, A., Hattori, M., Hayashi, T. (2008). The Whole-genome sequencing of the obligate intracellular bacterium *Orientia tsutsugamushi* revealed massive gene amplification during reductive genome evolution. DNA Res. *15*, 185-199.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

Technical Staff	KAJIURA-KOBAYASHI, Hiroko
NIBB Research Fellow	ICHIKAWA, Takehiko
Visiting Scientist	KANDA, Rieko
Technical Assistant	OKA, Naomi
Secretary	KAMIYA, Akemi

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).

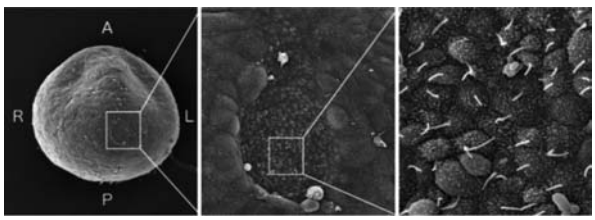


Figure 1. Left: ventral view of a 7.5-day mouse embryo. Middle: the node. Right: node cilia.

The leftward flow, called nodal flow, determines subsequent L-R development. This principle has been confirmed by our experiments, which demonstrated that embryos raised in a rightward artificial flow of culture medium develop reversed L-R asymmetry (Figure 2; Nonaka et al., 2002).

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

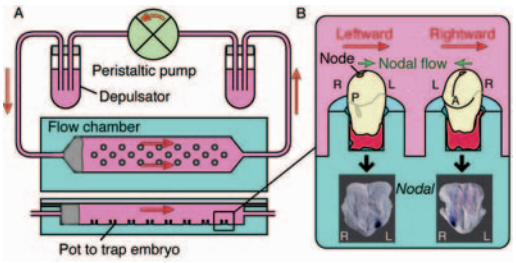


Figure 2. Flow culture exiperiment. A) A peristaltic pump and depulsators supply constant fluid flow in the chanber (red arrowheads). B) Embryos held in the pots receive the pump-driven flow on their surface. If the pump-driven flow reverses the intrinsic nodal flow, expression of nodal, a master gene for leftness, is reversed (Right).

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 phototoxity and the limitations of deep imaging. The Digital Scanned Light-sheet Microscope (DSLM, Figure 3) 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 set of DSLM and started to visualize whole mouse embryo at gastrulating stages with single cell resolution.

Additionally, we support researchers who are interested in using our DSLM and two-photon microscope. Several collaborative projects are in progress.

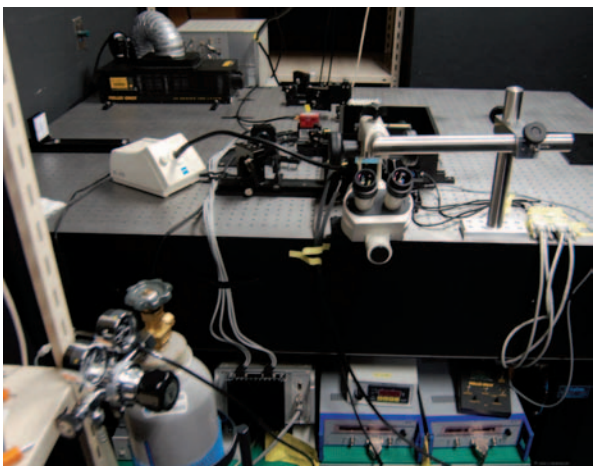


Figure 3. DSLM on a optical table.

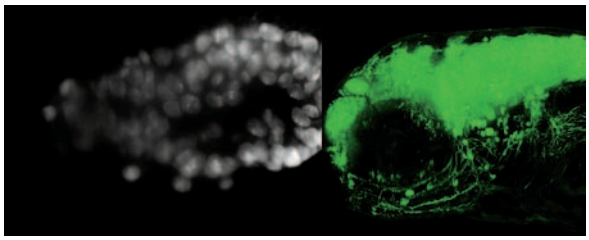


Figure 4. Images taken with DSLM. Left: optical section of a 6-day mouse embryo with fluorescent nuclei. Right: a juvenile zebrafish head expressing GFP in neurons.

STRATEGIC PLANNING DEPARTMENT

Chair
UENO, Naoto

Associate Professor	KODAMA, Ryuji
Assistant Professor (Specially appointed)	KURATA, Tomoko
NIBB Research Fellow	KURATA, Tomoko*
Technical Assistants	MUKOHDA, Yasuyo OTA, Misaki MAEDA, Sanae TANAKA, Megumi OTA, Kyoko EMDE, Jason R.

The Strategic Planning Department was founded in April 2005 as a central office for the efficient management of cooperational activities with other organizations, the distribution of scientific information to the public, planning and managing conferences, workshops and other extramural activities. The department also assists the director-general in preparing for NIBB's evaluation procedure and in planning a long-range strategy for the institute.

The main activities of the Department in 2008

1) Supporting international conferences

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

2) Management of education-related programs

Internship Program 2008

The Second and Third International Practical Course (Mar. and June, 2008)

3) Press Release

News on scientific achievements are sent to newspaper and magazine reporters via leaflets, e-mails and password protected web pages. For some of the releases, we arranged a press conference.

4) Editing of publications

Bulletin of NIBB 2008 (in Japanese, in preparation)

Annual Report of NIBB 2008 (in English, with proof reading)

5) Production of posters and leaflets

Design and distribution of posters for international conferences and advertisements for the graduate school's entrance examination (Figure 1)

6) Updating and maintenance of NIBB web page

7) Publication of "NIBB News"

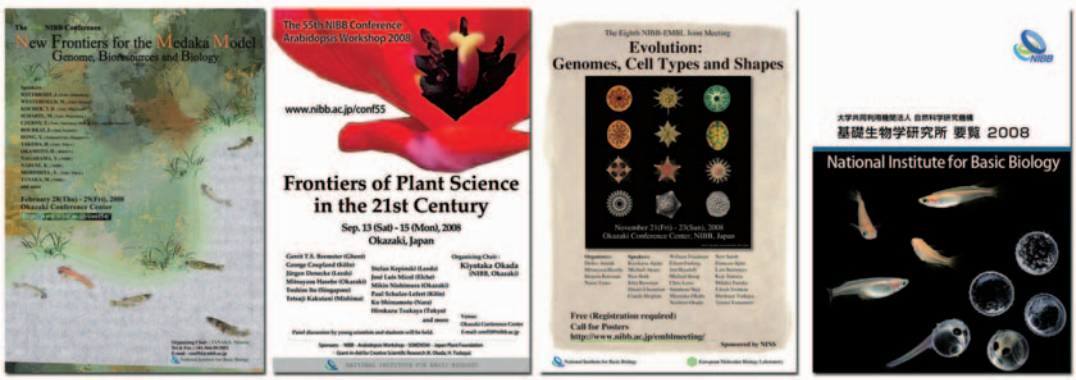
(Intra-institutional newsletter in Japanese)

8) Maintenance of the achievement archives of NIBB

9) Assisting visitors (in collaboration with Technical Division)

Name	*Date*	*Title*	*Organizer*
54th NIBB Conference	February 28-29	New Frontiers for the Medaka Model -Genome, Bioresources and Biology-	M. Tanaka
55th NIBB Conference	September 13-15	Frontiers of Plant Science in the 21st Century	K. Okada
8th NIBB-EMBL Joint Meeting	November 21-22	Evolution: Genomes, Cell Types and Shapes	D. Arendt M. Hasebe S. Kuratani N. Ueno

Table 1. International conferences managed by Strategic Planning Department in 2008



54th NIBB Conference | 55th NIBB Conference | 8th NIBB-EMBL Meeting | Bulletin of NIBB 2008 (in Japanese)

Figure 1. Examples of posters, pamphlets and abstract books produced by the Strategic Planning Department in 2008

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

Research Support Facilities

Head
TAKADA, Shinji

● Large Spectrograph Laboratory

Professor (Adjunct)	WATANABE, Masakatsu
Technical Staff	HIGASHI, Sho-ichi
Technical Assistant	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.

Publication List on OLS Collaboration

〔Original papers〕

- Ikehata, H., Kawai, K., Komura, J., Sakatsume, K., Wang, L., Imai, M., Higashi, S., Nikaido, O., Yamamoto, K., Hieda, K., Watanabe, M., Kasai, H., and Ono, T. (2008). UVA1 genotoxicity is mediated not by oxidative damage but by cyclobutane pyrimidine dimers in normal mouse skin. J. Invest. Dermatol. *128*, 2289-2296.
- Ioki, M., Takahashi, S., Nakajima, N., Fujikura, K., Tamaoki, M., Ioki, M., Takahashi, S., Nakajima, N., Fujikura, K., Tamaoki, M., Saji, H., Kubo, A., Aono, M., Kanna, M., Ogawa, D., Fukazawa, J., Oda, Y., Yoshida, S., Watanabe, M., Hasezawa, S., and Kondo, N. (2008). An unidentified ultraviolet-B-specific photoreceptor mediates transcriptional activation of the cyclobutane pyrimidine dimer photolyase gene in plants. Planta *229*, 25-36.
- Mori, E., Takahashi, A., Kitagawa, K., Kakei, S., Tsujinaka, D., Unno, M., Nishikawa, S., Ohnishi, K., Hatoko, M., Murata, N., Watanabe, M., Furusawa, Y., and Ohnishi, T. (2008). Time course and spacial distribution of UV effects on human skin in organ culture. J. Radiat. Res. *49*, 269-277.
- Nagai, Y., Miyagishi, D., Akagawa, T., Ohishi, F., Ueno, H., Kobayashi, K., Yamashita, K., and Watanabe, J. (2008). Photodegradation mechanisms in poly(2,6-butylenenaphthalate-co-tetramethyleneglycol) (PBN-PTMG), Part III: Photodegradation induced by the carbonyl group in n, π* excited states. Polym. Degradat. Stabil. *93*, 134-138.
- Nagao, A., Zhao, X., Takegami, T., Nakagawa, H., Matsui, S., Matsunaga, T., and Ishigaki, Y. (2008). Multiple shRNA expressions in a single plasmid vector improve RNAi against the XPA gene. Biochem. Biophys. Res. Commun. *370*, 301-305.
- Simamura, E., Shimada, H., Ishigaki, Y., Hatta, T., Higashi, N., and Hirai, K-I. (2008). Bioreductive activation of quinone anti-tumor drugs by mitochondrial voltage-dependent anion channel 1. Anat. Sci. Int. *83*, 261-266.
- Suzuki, T., Takashima, T., Izawa, N., Watanabe, M., and Takeda, M. (2008). UV radiation elevates arylalkylamine N-acetyltransferase activity and melatonin content in the two-spotted spider mite, *Tetranychus urticae*. J. Insect Physiol. *54*, 1168-1174.

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; 1.6TBx10), 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, four 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.

RESEARCH CENTER FOR INTEGRATIVE AND COMPUTATIONAL BIOLOGY

Head
HASEBE, Mitsuyasu

Professor (Concurrent) MOCHIZUKI, Atsushi
Secretary UMEBAYASHI, Hiromi

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. A 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

In April of 2008 we held an international meeting on systems biology in Barcelona as a collaborative program with EMBL. We had many participants studying biological systems using different methods, including physics, mathematics and computational science. The meeting enhanced interactions between researchers in different fields and resulted in some collaborative works between them.

CENTER FOR TRANSGENIC ANIMALS AND PLANTS

Head
IGUCHI, Taisen

Associate Professors	WATANABE, Eiji
	SASAOKA, Toshikuni
	TANAKA, Minoru
Technical Staff	HAYASHI, Kohji
	NOGUCHI, Yuji
	ICHIKAWA, Yoko
	TAKAGI, Yukari
Supporting Staff	YASUDA, Mie
	KAWAMURA, Motofumi
	OKUDA, Tadayoshi
	INADA, Yosuke
	AJIOKA, Rie
	NAKASHIMA, Machiko
	YAMANAKA, Megumi
	WATANABE, Kaori

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 2008 (from January 1 to December 31) 3,509 mice and 1,126 fertilized eggs were brought into the CTAP in the Yamate area, and 37,137 mice (including pups bred in the facility) and 120 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 the *in vitro* fertilization-

embryo transfer techniques, and stored using cryopreservation.

A new mouse facility in the Myodaiji area opened at the beginning of 2005. The facility provides research-supporting activities to researchers in the Myodaiji area. In 2008 (from January 1 to December 31) 11 mice were brought into the CTAP in the Myodaiji area, and 257 mice (including pups bred in the facility) were taken out.

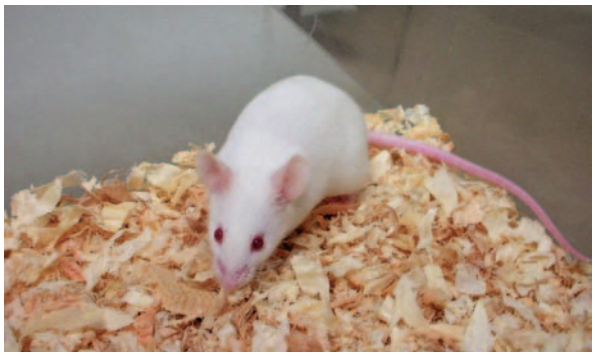


Figure 3. A laboratory mouse

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 a 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 transgenic animals.

In 2008 (from January 1 to December 31), 10,442 medaka and zebrafish (124 eggs, 3,761 embryos and 6,557 adults) were brought to the facility and 82,929 medaka and zebrafish (75,521 fertilized eggs, 6,454 embryos and 954 adults, including animals bred in the facility) were taken out. In a laboratory for chick embryos 7,070 fertilized chicken eggs were brought in and 154 fertilized eggs and 10 chicken embryos were taken out. These animals were used for research activities in neurobiology and developmental biology.

In 2007 NIBB was selected as a core facility of the National BioResource Project of Medaka. We have supported the activities of NBRP Medaka by providing standard strains, induced mutants and transgenic lines and training personnel regarding fish maintenance.

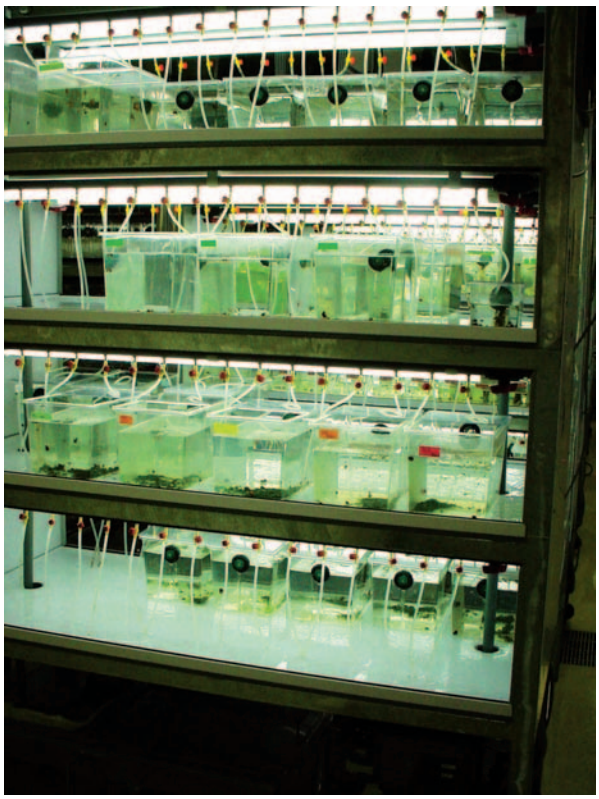


Figure 4. Breeding equipment for small fish

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 the brain sensing system for body fluid water and sodium homeostasis using gene-targeting mice. 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.

CENTER FOR ANALYTICAL INSTRUMENTS (managed by NIBB)

Head of Facility
IIDA, Shigeru

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

The Center is responsible for amino acid sequence analysis, amino acid analysis, and chemical syntheses of peptides, 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-100Σ, PI-50α, PI-50, PI-200)
Automatic Nucleic Acid Isolation System (Kurabo NA-2000)
Genetic Analyzers (ABI PRISM 3130xl, 310)
Thermal Cyclers (Perkin Elmer PJ-9600, Takara TP-300, 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)
Microscopes (Carl Zeiss Axiophot, Axiovert)
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

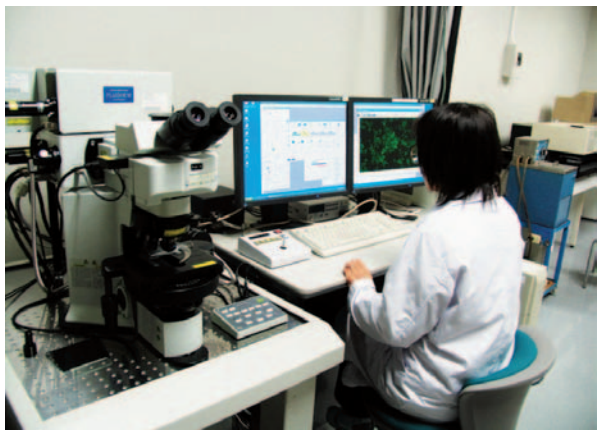


Figure 2. Confocal Laser Scanning Microscope

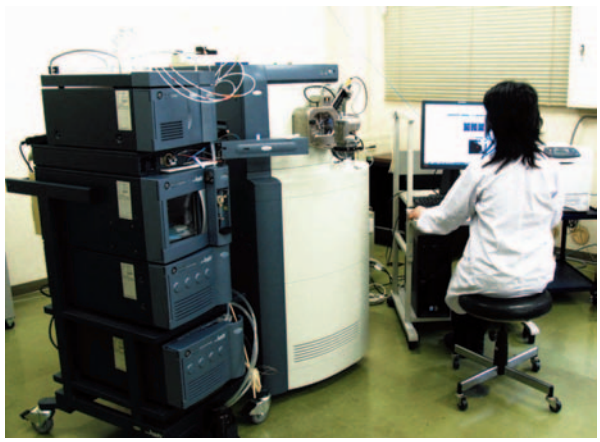


Figure 3. LC/Q-TOF-MS

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
ISHIKAWA, Azusa

● Center for Analytical Instruments

Unit Chief MORI, Tomoko
Subunit Chief MAKINO, Yumiko
YAMAGUCHI, Katsushi
Technical Staff TAKAMI, Shigemi
OKA, Sanae
Technical Assistants 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
Subunit Chief KABEYA, Yukiko

● Developmental Biology

Technical Staff TAKAGI, Chiyo
UTSUMI, Hideko
NODA, Chiyo

● Neurobiology

Unit Chief OHSAWA, Sonoko
Subunit Chief TAKEUCHI, Yasushi

● Evolutionary Biology and Biodiversity

Unit Chief FUKADA-TANAKA, Sachiko
Technical Staff MOROOKA, Naoki
SUMIKAWA, Naomi

● Environmental Biology

Subunit Chief MIZUTANI, Takeshi

Reception

Supporting Staff SAKAGAMI, Mari
TSUZUKI, Shihoko
KATAOKA, Yukari
UNO, Satoko
MAEDA, Yumi
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.

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor	OGAWA, Kazuo *(Radiation Protection Supervisor)*
Technical staff	MATSUDA, Yoshimi *(Radiation Protection Supervisor)*
	SAWADA, Kaoru
	IINUMA, Hideko
Supporting staff	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 2008 to December 2008 are presented in Table 1.

Users counted by the monitoring system going in and out of the controlled areas numbered 4,151 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 floor and the waste water system have deteriorated. Figure 3 shows the repair work done on Room 9 of the CFBI-branch to accommodate the law.

	Myodaiji-Area	Yamate-Area
registrants	145	103
users	66	35

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2008

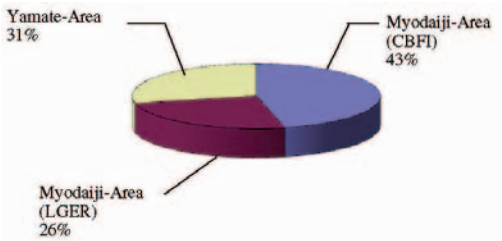


Figure 1. Percentage of users going in and out of each controlled area in 2008

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch		
users	1603	964	1038	3605
visitors	319	80	147	546
total	1922	1044	1185	4151

Table 2. Users and visitors who entered each controlled area in 2008

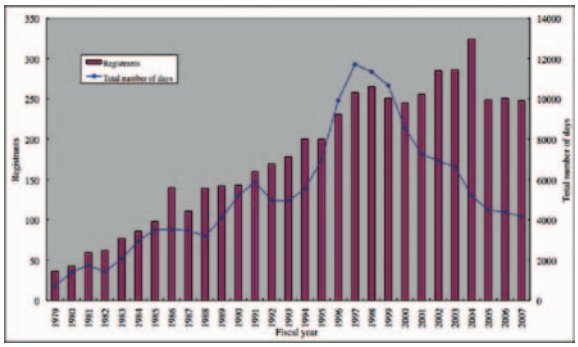


Figure 2. Annual changes of registrants and number of totals per fiscal year

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch		
^{125}I Receive	167	0	370	537
^{125}I Used	167	0	370	537
^{45}Ca Receive	37000	0	-	37000
^{45}Ca Used	3000	0	-	3000
^{35}S Receive	94000	0	0	94000
^{35}S Used	8400	0	0	8400
^{32}P Receive	179500	555500	254250	989250
^{32}P Used	87000	414320	203480	704800
^{14}C Receive	12025	0	0	12025
^{14}C Used	8604	0	0	8604
^{3}H Receive	638250	0	8325056	8963306
^{3}H Used	91150	0	7400056	7491205

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2008

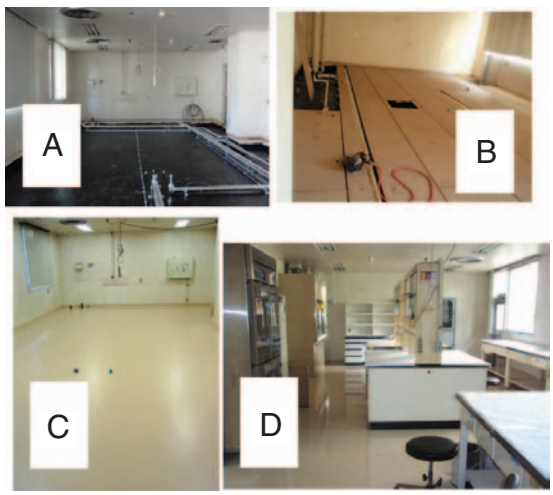


Figure 3. Repair work on room 9
A: Floor painting (waterproofing)
B: Floor construction
C: Closing floor sheet
D: Completion

54th NIBB Conference
New Frontiers for the Medaka Model – Genome, Bioresources and Biology

Organizing Chair : Minoru Tanaka

February 28 (Thu)-29 (Fri), 2008

The medaka is an emerging model vertebrate and is beginning to aid the exploration of new fields of biology. The symposium was the first international symposium after the core facility of NBRP medaka was transferred to and established at NIBB and the entire genomic sequence of medaka was published. The symposium focused on not only a variety of biological studies but also on the genome and bioresources. First rate scientists from Japan, Asia, Europe and America gathered at the symposium and surveyed the biology of medaka, the novel skills that support the biology, the genomic information infrastructure and a variety of medaka resources, and discussed the future of such studies. Although the symposium did not set up a poster presentation for young scientists and students, more than 100 researchers participated in the conference.

The presentations on the basic sciences covered wide areas of biology, from the fundamental issues of development, evolution and genome research to the analysis of behavior. This represents one feature of medaka biology, together with its application to environmental assessment and toxicology. It is noteworthy that most of these studies were based on or were closely associated with genomic infrastructures and novel technologies that have recently become available for the researchers.

The symposium attracted considerable attention from a wide audience and was reported in the May issue of "nature DIGEST", vol.5 2008. Several speakers from the symposium were interviewed by a reporter in the article.

Lastly, I'd like to briefly mention that this was the first NIBB conference that accepted donations from several overseas companies. An unexpectedly wide and high level of attention to and interest in biology using medaka in the scientific community was indicated by the response.

(Minoru Tanaka)

Speakers

CHENG, Shuk Han (City Univ. Hong Kong, China SAR), CZERNY, Thomas (Univ. Applied Sciences, Austria), HONG, Yunhan (NUS, Singapore), JOLY, Jean Stephane (INRA/CNRS, France), SCHARTL, Manfred (Univ. Wuerzburg, Germany), TSAI, Huai-Jen (National Taiwan Univ., Taiwan), WESTERFIELD, Monte (Univ. Oregon, USA), WINKLER, Christoph (NUS, Singapore), WINN, Richard N. (Univ. Georgia, USA), WITTBRODT, Joachim (Univ. Heidelberg / EMBL, Germany)

HAMAGUCHI, Satoshi (Niigata Univ., Japan), IWANAMI, Norimasa (Univ. Tokushima, Japan), KAWAMURA, Shoji (Univ. Tokyo, Japan), KIKUCHI, Kiyoshi (Univ. Tokyo, Japan), KUDO, Akira (Tokyo Inst. Tech., Japan), MORISHITA, Shinichi (Univ. Tokyo, Japan), NAGAHAMA, Yoshitaka (NIBB, Japan), NARUSE, Kiyoshi (NIBB, Japan), NISHINA, Hiroshi (Tokyo Med. & Dental Univ., Japan), OKAMOTO, Hitoshi (RIKEN BSI, Japan), OKUBO, Kataaki (NIBB, Japan), SASADO, Takao (Nagahama Inst. Bio-Sci. & Technology, Japan), TAKEDA, Hiroyuki (Univ. Tokyo, Japan), TAKEUCHI, Hideaki (Univ. Tokyo, Japan), TANAKA, Minoru (NIBB, Japan), TANIGUCHI, Yoshihito (Kyoto Univ., Japan), WAKAMATSU, Yuko (Nagoya Univ., Japan), YAMASHITA, Masakane (Hokkaido Univ., Japan), YAMAZAKI, Yukiko (NIG, Japan)

55th NIBB Conference
Frontiers of Plant Science in the 21st Century

Organizing Chair : Kiyotaka Okada

September 13 (Sat)-15 (Mon), 2008

For three days in mid-September 2008, from the 13th to the 15th, 147 people gathered in the Okazaki Conference Center and discussed the present and future of Plant Science under the title of "Frontiers of Plant Science in the 21st Century". The topics ranged from cellular, developmental, and genetic biology to systems biology, evolutional biology and engineering. The program of the conference can be found on our home page at http://www.nibb.ac.jp/conf55. We had a special panel discussion on the afternoon of the 14th, which was organized and held by a group of our graduate students as part of their Graduate Student Education Program sponsored by the Graduate University for Advanced Studies (SOKENDAI).

After the conference, we asked several attendants to give us a frank review of the aims and execution of the conference. The comments and advice that we received will be a great help to us in improving future NIBB conferences and can be found below.

(Kiyotaka Okada)

Invited Speakers

BEEMSTER, Gerrit T.S. (Ghent Univ., Belgium), CAUSIER, Barry (Univ. Leeds, UK), COLLINS, Richard M. (Univ. Leeds, UK), COPPENS, Frederik (Ghent Univ., Belgium), COUPLAND, George M. (Max Planck Inst., Germany), GIAKOUNTIS, Antonis (Max Planck Inst., Germany), GONEHAL, Venugopala Reddy (UC, Riverside, USA), ITO, Toshiro (Temasek Life Sciences Lab., Singapore), KEPINSKI, Stefan (Univ. Leeds, UK), KNOX, Paul (Univ. Leeds, UK), MICOL, Jose Luis (Univ. Miguel Hernandez , Spain), PEREZ-PEREZ, Jose M. (Univ. Miguel Hernandez & Inst. Bioingenieria, Spain), SAIJO, Yusuke (Max Planck Inst., Germany), SCHULZE-LEFERT, Paul (Max Planck Inst., Germany), SUN, Bo (Temasek Life Sciences Lab., Singapore)

ASHIKARI, Moto (Nagoya Univ., Japan), BREUER, Christian (RIKEN Plant Sci. Center, Japan), EZURA, Hiroshi (Univ. Tsukuba, Japan), HARA-NISHIMURA, Ikuko (Kyoto Univ., Japan), HASEBE, Mitsuyasu (NIBB, Japan), KAKUTANI, Tetsuji (NIG, Japan), KAWAGUCHI, Masayoshi (Univ. Tokyo, Japan), MACHIDA, Yasunori (Nagoya Univ., Japan), MATSUBAYASHI, Yoshikatsu (Nagoya Univ., Japan), NISHIMURA, Mikio (NIBB, Japan), OKADA, Kiyotaka (NIBB, Japan), SHIMAMOTO, Ko (NAIST, Japan), TAKEDA, Seiji (NAIST, Japan), TANAKA, Yoshi (Suntory Ltd, Japan), TSUKAYA, Hirokazu (Univ. Tokyo / NIBB, Japan), YAMAGUCHI, Takahiro (NIBB, Japan)

Organizing Committee

OKADA, Kiyotaka (NIBB), TSUKAYA, Hirokazu (Univ. Tokyo / NIBB), NISHIMURA, Mikio (NIBB), HASEBE, Mitsuyasu (NIBB), YAMAGUCHI, Takahiro (NIBB)

55th NIBB Conference Frontiers of Plant Science in the 21st Century

Comments from the participants

Dr. Antonis Giakountis

Max Planck Institute for Plant Breeding Research, Germany.

The organizers did an excellent job, on multiple levels, of preparing the NIBB55 conference. The invited speakers were certainly chosen wisely and in such a way that all of the sessions were interesting. In terms of timing the conference was also successful and there were no significant deviations from the schedule. One suggestion would be to offer the opportunity to selected poster presenters to take 20 minutes instead of 15 minutes for their talks, especially since the discussion was also included in that time. Perhaps the starting talk of each session could be reduced to 30-35 minutes instead of 40 minutes so as to provide this extra time. I appreciated and enjoyed the politeness of the hosts and the Japanese students a lot and there were many opportunities for social interaction at the conference. Furthermore it was a very interesting idea to have a panel discussion conducted exclusively by young scientists on topics that the students themselves had selected. This certainly made this session very current and up-to-date. An additional suggestion would be to perhaps split future panel discussions up over two days or to move them to morning sessions so as to stimulate even more discussion. In conclusion, it was a very successfully and fruitful meeting which highlighted research opportunities in Japan as being very attractive.

Dr. Stefan Kepinski

Centre for Plant Sciences, University of Leeds, UK.

I thought the meeting was a wonderful success. Firstly, the selection of speakers and the quality of the presentations were excellent, making the meeting extremely satisfying scientifically and intellectually. The broad theme of "Frontiers of plant science in the 21st century" worked very well and I imagine drew wide interest from across the diverse research activity of NIBB. If you were to consider more focused meetings in the future then I would suggest themes that still allow wide participation across your institute and throughout Japan. Examples could be 'Systems biology and quantitative modeling' or 'Single-cell-level biology' or 'Next generation sequencing' because although they are focused on particular approaches, they are useful in showing how these new approaches can boost the research capacity of a whole range of groups.

I thought that it was an excellent idea to let the younger students organize the presentation and discussion session on the future of plant research and I very much enjoyed participating in the discussions. I also enjoyed the fact that there was time to chat with other scientists both at coffee breaks and at the poster sessions. The only thing you might follow up on is that the younger participants felt there was enough time to 'pluck up the courage' to approach the speakers to talk about their work. At interesting meetings like this it can often be the case that the speakers and senior scientists are so engaged with the science and talking to their colleagues that it can be difficult for the younger scientists to find the time to talk to them.

With respect to enhancing opportunities for younger researchers to interact with most established PIs and visiting speakers, the only suggestion I can make is that you could consider setting up a lunch where each PI dined with a small group of, say, 4-5 students to allow them time to talk about life in science and research in general. As alluded to above, it can often be daunting for early career scientists to approach older colleagues and this might be a useful way of helping them overcome any shyness in this regard. Having such an event early in the meeting would probably be most useful as it would allow them to get to know the visiting and senior scientists straight away and make them feel more confident about continuing discussions throughout the meeting.

All in all it was a really very enjoyable and stimulating meeting. It was very well organized and ran very smoothly. I'd like to thank you and the organizers again for both the invitation and your excellent hospitality while we were with you in Okazaki.

Dr. Jose Manuel Perez-Perez

Universidad Miguel Hernandez, Spain.

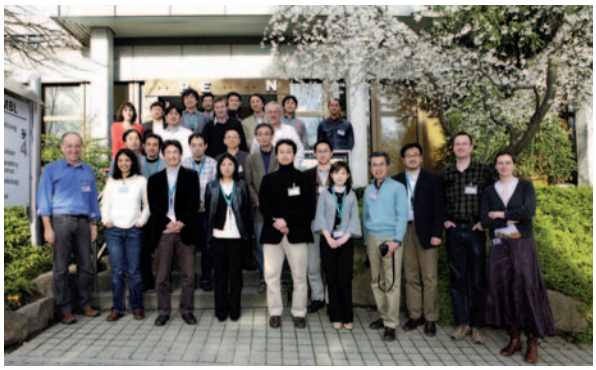
The 55th NIBB Conference Arabidopsis Workshop 2008 entitled "Frontiers of Plant Science in the 21st Century" was held in Okazaki during September 13th-15th and chaired by NIBB Director Prof. Okada and others. The workshop consisted of 25 conferences (15 of which were from foreign researchers invited from outside Japan) arranged in 9 scientific sessions covering most plant topics. Fifty-two posters from Japanese researchers were presented, with nine of them being selected for oral discussions. The location of the posters (opposite the conference hall) helped encourage discussion with the authors at any time and not only during the poster sessions. About 130 participants, most of them in the initial stages of their careers (PhD students and postdocs), attended the meeting, which was nicely organized and properly scheduled at the Okazaki conference centre (OCC). The meeting was the right size to allow for a fruitful flow of information and to encourage eventual collaborations. I personally find it very useful for the young PhD students to attend and participate in this kind of meeting because it provides them with a good overview of the latest plant research and allows them plenty of time to discuss their favorite research topic with their colleagues. The conference hall was very appropriate for the presentations, with very comfortable chairs and good facilities. Lunch at the conference centre was quite diverse and copious with enough refreshment time after each scientific session. Furthermore, get-together dinners with our Japanese hosts (PhD and postdocs in one case and Professors in the others) were very enjoyable, not only for the food but also for the relaxed and friendly environment. In this 55th NIBB edition, the students organized a session about the future of plant research that began after short introductions by the invited young researchers. Although the aim of this panel discussion was to enhance participation from the young students in the audience, it was mostly the senior PIs who participated. In my opinion, the topic list chosen by the students was too broad. Maybe focusing only on a few hot topics (the future of transgenic plants, climate change, etc.) and building a real debate table around them would have helped to improve audience participation. Although most of the talks I attended were not related to my main research topic (leaf development in Arabidopsis), all of the presentations were clear and concise with a definite time for enough questions too. Maybe it would help the students if they were able to ask their questions in Japanese first, with an English translation afterwards. I really liked that most of the speakers presented unpublished or ongoing work from their laboratories, which gives a good impression about the kind of science that's being performed nowadays in Japan. The travel and accommodation for the invited speakers was handled very efficiently, and I did not have to worry at all about flight tickets, etc., since everything was arranged in advance from Japan. This meeting has been my first contact with Japanese culture and as far as I can say the most interesting one. I am looking forward to the opportunity to come back again in 2010!

NIBB-EMBL JOINT MEETINGS

The 6th NIBB-EMBL Meeting: "Evolution of Epigenetic Regulations"

March 17 (Mon)-19 (Wed), 2008 | EMBL, Heidelberg

One of the most important questions in biology is how gene activity is temporally and spatially controlled. It has been several decades since we learned that not only DNA sequences but also other aspects of genetic regulation such as DNA/protein modifications influence chromatin remodeling and gene expression and thus the development of a variety of organisms. Epigenetic regulation involving methylation and acetylation of DNA or histone protein appears to be evolutionary conserved among species. However, it also seems that each organism evolves its own mechanism of epigenetic regulation of gene activity as shown by the fact that bacteria such as E. *coli*, yeast, the nematode, and the fruit fly lack DNA methylation. As organizers, Jürg Müller (EMBL), Kunio Shiota (Univ. Tokyo), and Shigeru Iida (NIBB) arranged this meeting in order to exchange the latest information on transcriptional regulation and remodeling of chromatin structure, regulation of cell differentiation, genomic imprinting, and so on. The sessions enabled discussion about both epigenetic regulation at different levels as well as the evolutional conservation of the epigenetic mechanism. As a result, species-specific mechanisms were highlighted through a series of presentations with a variety of organisms from yeast, plants, arthropods, to mammals. In addition, this meeting emphasized that genome-wide approaches to understanding the epigenetic state of the whole genome is important. We thank Drs. David Lane and Shigeru Iida for their stimulating plenary lectures.



Speakers

AKHTAR, Asifa (EMBL) , ALLSHIRE, Robin (Welcome Trust Univ. Edinburgh), LADURNER, Andreas (EMBL), MOSHER, Becky (The Sainsbury Laboratory), MUELLER, Juerg (EMBL), MUELLER, Christoph (EMBL), PASZKOWSKI, Jerzy (Univ. Geneva), REINBERG, Danny (HHMI at NYU School of Medicine), STANCHEVA, Irina (Univ. Edinburgh), HAMADA, Kyoko (RIKEN CDB), HIROSE, Susumu (National Institute of Genetics), IIDA, Shigeru (NIBB), IMAMURA, Takuya (Kyoto Univ.) , ISHINO, Fumitoshi (Tokyo Med. & Dent. Univ.), KAKUTANI, Tetsuji (National Institute of Genetics), MATSUI, Yasuhisa (Tohoku Univ.), NAKAYAMA, Jun-ichi (RIKEN CDB), OHGANE, Jun (Univ. Tokyo), SASAKI, Hiroyuki (National Institute of Genetics), SHINKAI, Yoichi (Kyoto Univ.), SHIOTA, Kunio (Univ. Tokyo), TANAKA, Satoshi (Univ. Tokyo)

The 7th NIBB-EMBL Meeting: "Systems Biology and Functional Genomics"

April 18 (Fri)-19 (Sat), 2008 | Center for Genomic Regulation, Barcelona

Center for Genomic Regulation (CRG) is located at the Barcelona Biomedical Research Park (PRBB), one of the largest research clusters in southern Europe. This meeting was held at the new CRG/PRBB building overlooking the beautiful Catalonian coast. The main purpose of the meeting was to bring Japanese and European scientists in the field together to grasp the current status of systems biology and functional genomics, and to discuss the future direction of the field. We also aimed to stimulate international collaborations between scientists. The meeting included excellent presentations on gene and protein networks, biological systems controlling higher-order phenomena such as complex morphogenesis of organs of animals and plants, and gene regulatory networking of circadian rhythm. As a result of the meeting we realized some key issues that need to be addressed are the setting-up of experimental systems by which data with minimum noise can be obtained,

comprehensive collection of data sets with different conditions or genetic backgrounds, a well-designed presentation of data that allows the extraction of biological significance, and the necessity of mathematical modeling to predict the principles behind biological phenomena. There is an ever increasing amount of biological data being accumulated by the achievements of large-scale biology such as genomic, proteomics, and phenomics, and therefore we certainly need sophisticated "Systems Biology" to make the best use of them.

Organizers of this meeting were Luis Serrano (EMBL/CRG), Eileen Furlong (EMBL), and Atsushi Mochizuki (NIBB).

Speakers

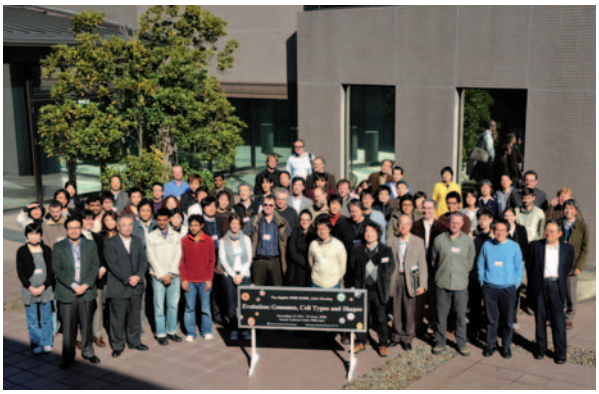
FURLONG, Eileen (EMBL), HUFNAGEL, Lars (EMBL), ISALAN, Mark (CRG), LEHNER, Ben (CRG), LEMAIRE, Patrick (IBDM), NEDELEC, Francois (EMBL), OLIVERI, Paola (UCL), PAPONOV, Ivan (Univ. Freiburg), RUSSELL, Rob (EMBL), SHARPE, James (CRG), STEINMETZ, Lars (EMBL), WITTBRODT, Jochen (EMBL)
HORIKAWA, Kazuki (Hokkaido Univ.), ISHIHARA, Shuji (Univ. Tokyo), ITO, Takashi (Univ. Tokyo), KURODA, Shinya (Univ. Tokyo), MIURA, Takashi (Kyoto Univ.), MOCHIZUKI, Atsushi (NIBB), SATOU, Yutaka (Kyoto Univ.), SHIGENOBU, Shuji (NIBB), UEDA, Hiroki (RIKEN, CDB)

The 8th NIBB-EMBL Meeting: "Evolution: Genomes, Cell Types and Shapes"

November 21 (Fri)-23 (Sun), 2008 | Okazaki Conference Center, Okazaki

This meeting was planned by three organizers, Detlev Arendt (EMBL), Mitsuyasu Hasebe (NIBB) and Shigeru Kurtani (RIKEN, CDB), to address current problems in evolutionary biology at different hierarchical levels of animal and plant life. The levels discussed included genomes, gene regulatory networks at the micro-level and organelles, cell types, organs, and morphology of species at the macro-level. Among the many meetings in evolutionary biology meetings, in which typically only a few limited topics of similar levels are discussed, this meeting was unique in that it overviewed the evolution of organismal life and brought the interconnectivity of the logic underlying different layers of evolutional events into focus. The life and evolutionary strategies of a variety of organisms, from single-celled organisms such as bacteria and yeast to higher plants and vertebrate including mammals, were presented and discussed not only with mechanistic insights but also from the aspect of their impact on the history of organismal life.

Four graduate students from EMBL were invited to contribute to the meeting and presented their work. They also had chances to meet with graduate students of NIBB and exchange thoughts on their scientific life at each institution.



Speakers

AKAM, Michael (Univ. Cambridge), ARENDT, Detlev (EMBL Heidelberg), BORK, Peer (EMBL Heid.), BOWMAN, John (Monash Univ.), CHOURROUT, Daniel (Univ. Bergen), DESPLAN, Claude (New York Univ.), FRIEDMAN, William (Univ. Colorado), FURLONG, Eileen (EMBL Heid.), HASELOFF, Jim (Univ. Cambridge), HASTINGS, Nicola (EMBL Heid.), KNOP, Michael (EMBL Heid.), LIU, Ya-Hsin (EMBL Heid.), LOWE, Christopher (Univ. Chicago), SPITZ, Francois (EMBL Heid.), STEINMETZ, Lars (EMBL Heid.), TECHNAU, Ulrich (Univ. Vienna), TOMER, Raju (EMBL Heid.), TRACHANA, Kalliopi (EMBL Heid.)
AGATA, Kiyokazu (Kyoto Univ.), HASEBE, Mitsuyasu (NIBB), ISHINO, Fumitoshi (Tokyo Med. & Dental Univ.), KURATANI, Shigeru (RIKEN CDB), NOJI, Sumihare (Univ. Tokushima), OKABE, Masataka (The Jikei Univ. School of Med.), OKADA, Norihiro (Tokyo Inst. Tech.), SATOH, Nori (Kyoto Univ.), TAMURA, Koji (Tohoku Univ.), TANAKA, Mikiko (Tokyo Inst. Tech.), TSUKAYA, Hirokazu (Univ. Tokyo), UENO, Naoto (NIBB), YAMAMORI, Tetsuo (NIBB)

THE NIBB INTERNATIONAL PRACTICAL COURSE

The first NIBB International Practical Course was held in 2007 with the aim of providing young scientists around the world with opportunities to learn cutting-edge experimental skills and to communicate with experts in the field. The course replaced our Bioscience Training Course, which had been held for twenty years and which had, for the most part, only accepted participants from universities and institutes in Japan. The Second and Third International Practical Courses were held in 2008 as summarized below. The courses were given in a laboratory provided by NIBB and equipped with all 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 Second International Practical Course: "Developmental Genetics of Zebrafish and Medaka II"

- Period: March 3 (Sat)- 12 (Wed), 2008
- Participants: 12 (three from Hong Kong, two each from China and Taiwan, one each from Australia, Germany, Korea, Norway, and Japan)
- Lecturers:
  Dr. Shinichi Higashijima (National Institute for Physiological Sciences)
  Dr. Kiyoshi Naruse (NIBB)
  Dr. Kohei Hatta (University of Hyogo)
  Dr. Shoji Fukamachi (University of Konstanz)
- Research Results Presentations:
  Dr. Jochen Wittbrodt (University of Heidelberg)
  Dr. Kataaki Okubo (NIBB)
  Dr. Masahiko Hibi (RIKEN CDB)
  Dr. Mikiko Tanaka (Tokyo Institute of Technology)
  Dr. Koichi Kawakami (National Institute for Genetics)

Contents of the course: basic technologies, including gene knockdown by injecting anitisense morpholino oligo, as well as a number of advanced techniques such as BAC homologous recombination mediated transgenesis, genomic/bioinformatic techniques, and cell tracking using photoconvertible fluorescent proteins

The Third International Practical Course: "2008 NIBB Laboratory Course and Workshops on *Physcomitrella patens*"

- Period: June 30 (Mon)- July 4 (Fri), 2008
- Participants: 11 (six from Japan, two from America., and one each from Korea, Spain, and Sweden)
- Lecturers:
  Dr. Andrew C. Cuming (University of Leeds)
  Dr. Yasuko Kamisugi (University of Leeds)
  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*

Access

From Tokyo

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

From Central Japan International Airport

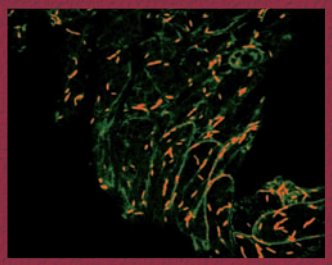
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

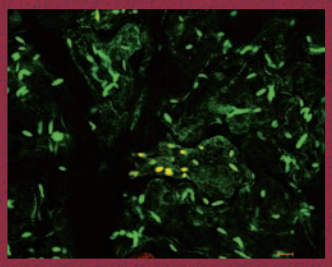
From Higashi Okazaki Station to Each Area

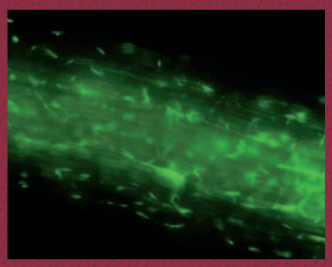
Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

By car

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







National Institutes of Natural Sciences
National Institute for Basic Biology

38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585 Japan
tel: +81 564-55-7000 fax: +81 564-53-7400
http://www.nibb.ac.jp/en/

Issued in March 2009
Edited by the Strategic Planning Department, NIBB