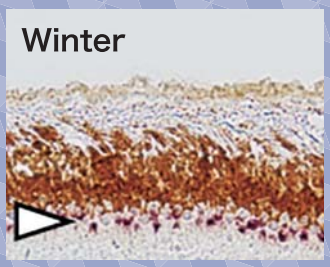
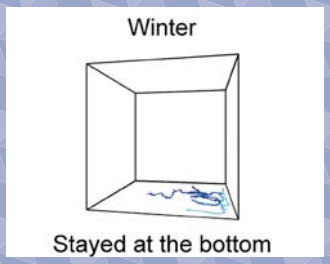
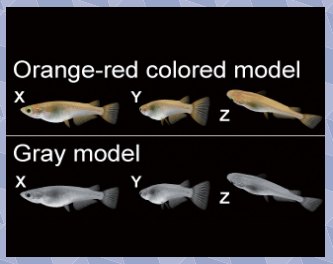
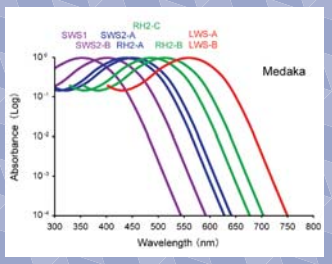
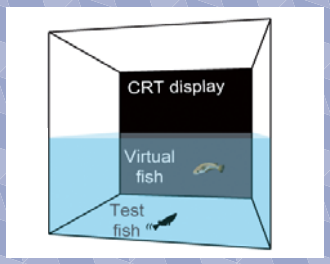
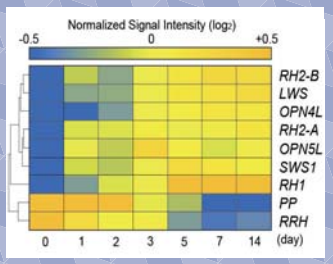


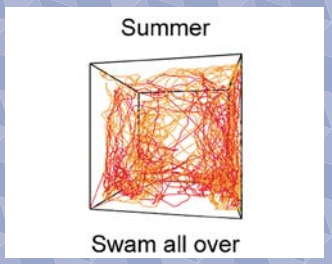


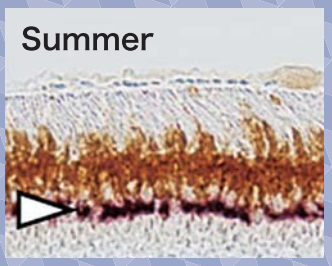












National Institute for Basic Biology

2017 ANNUAL REPORT

CONTENTS

The cover items are related to a paper titled "Dynamic plasticity in phototransduction regulates seasonal changes in color perception" (Shimmura et al., Nature Communications 2017) from the laboratory of Prof. Yoshimura. The paper demonstrated that color perception of Medaka, a small fish inhabiting rice fields and streams, varies greatly according to seasonal changes. See page 67 of this report for details.

INTRODUCTION

It is my great pleasure to introduce to you all the 2017 Annual Report of the National Institute for Basic Biology (NIBB), which outlines the research activities of the Institute and its effective function as a center for collaborative research in Japan over the last year. Given these important missions of the Institute, I recognize my strong responsibility to maintain the liberal and forward-looking atmosphere for research and discussion that NIBB has kept for many years, which I believe is a basis for these remarkable activities.

The winning of the 2016 Nobel Prize in Physiology or Medicine by Dr. Yoshinori Ohsumi, who is a Professor Emeritus of NIBB, was effective in reminding the society and the government of the importance of basic research. The government apparently compromised the trend to reduce the fundamental budget for universities and inter-university research institute corporations in total, and I sincerely wish we would be finally at a turning point to the increase of support, which would be indispensable to recovery of the leading status of Japan in science.

NIBB will stride properly to open up and foster new research fields in biology. To this end, every person in NIBB should do his/her best in accomplishing good research, keeping high ethics and compliance. Good science, even in a basic research field, will eventually benefit human beings. The history of science tells us this is true. NIBB will work hard to be truly acknowledged as a remarkable institution by society.

Please find in this booklet a summary of the research, collaborative, educational, and international activities of NIBB in 2017. I would like to note that we welcomed several new colleagues in 2017, including one associate professor, five assistant professors, and five NIBB research fellows, while four colleagues transferred to other institutes.

I would like to congratulate Associate Professor Nobuyuki Shiina for winning the Astellas Award for the Best Biomedical Research, Mr. Takuya Norizuki for winning an Outstanding Poster Award from an academic society and Dr. Takashi Matsuda for winning the 22nd Nagakura Research Incentive Award, as detailed on page 7.

To establish NIBB as an international leading institute in the field of basic biology, we always welcome your suggestions, comments and queries concerning our activities, in addition to your warm support.

Masayuki Yamamoto
Director General of NIBB
March 30, 2018

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

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

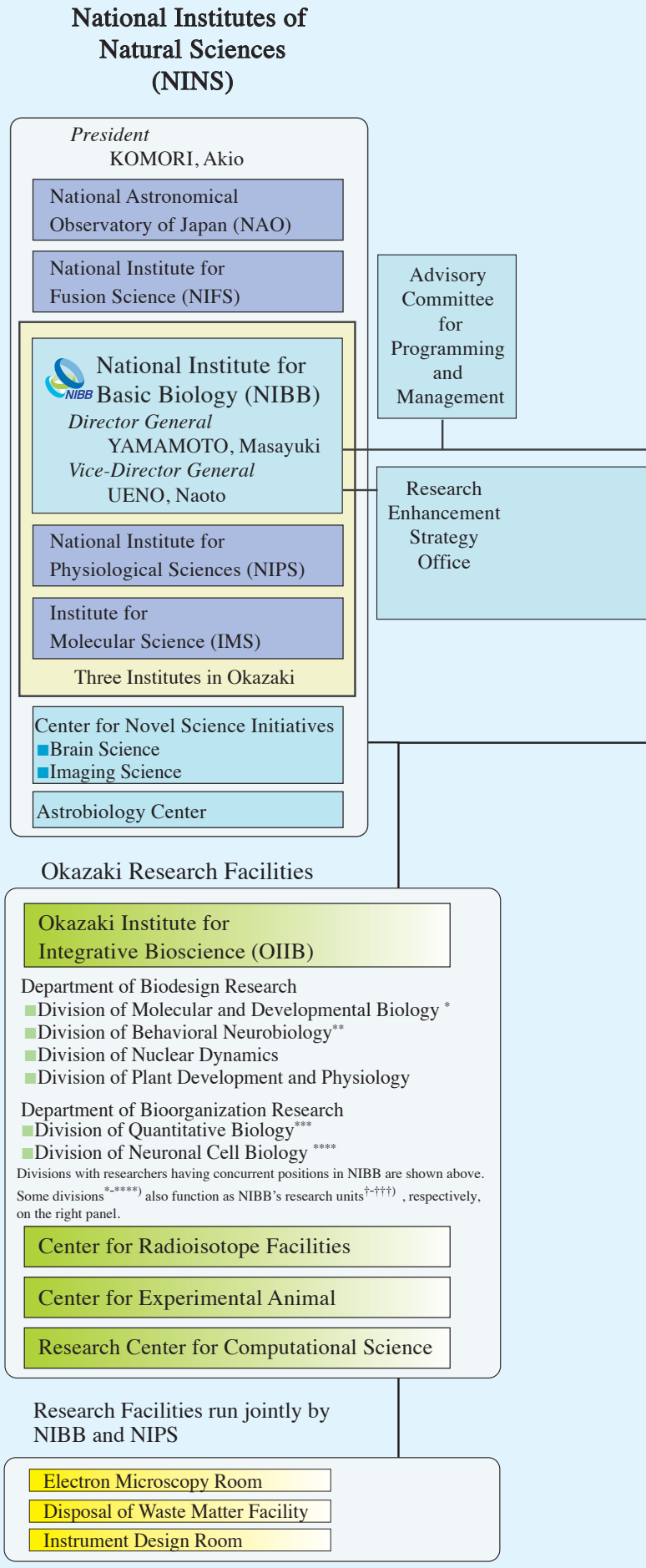
Policy, Decision Making, and Administration

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

The Research Enhancement Strategy Office, aimed at supporting researchers in order to improve NIBB's abilities as a collaborative research institution, was restructured in 2013 from the former Strategic Planning Department, Office of Public Relations, and Office of International Cooperation. The Office is made up of six groups (p. 90) and its activities are mainly carried out by URAs (University Research Administrators) in close collaboration with the NINS's Research Enhancement Promotion Headquarters.

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

Organization



National Institute for Basic Biology (NIBB)

As of January 1, 2018

Research Units

- **Cell Biology**
  - Division of Cellular Dynamics
  - Division of Quantitative Biology†††
  - Division of Chromatin Regulation
  - Laboratory of Cell Responses
  - Laboratory of Neuronal Cell Biology††††
  - Laboratory of Stem Cell Biology
- **Developmental Biology**
  - Division of Morphogenesis
  - Division of Molecular and Developmental Biology †
  - Division of Embryology
  - Division of Germ Cell Biology
- **Neurobiology**
  - Division of Molecular Neurobiology
  - Division of Behavioral Neurobiology††
  - Laboratory of Neurophysiology
- **Evolutionary Biology and Biodiversity**
  - Division of Evolutionary Biology
  - Division of Symbiotic Systems
  - Division of Evolutionary Developmental Biology
  - Laboratory of Bioresources
  - Laboratory of Morphodiversity
  - Laboratory of Biological Diversity
- **Environmental Biology**
  - Division of Environmental Photobiology
  - Division of Seasonal Biology (Adjunct)
- **Theoretical Biology**
  - Laboratory of Genome Informatics
- **Imaging Science**
  - Laboratory for Spatiotemporal Regulations

Research Support Facilities

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

- **Technical Division**
- **Section of Health and Safety Management**

- Evaluation and Information Group
- Public Relations Group
- International Cooperation Group
- Collaborative Research Group
- Young Researcher Support Group
- Gender Equality Promotion Group

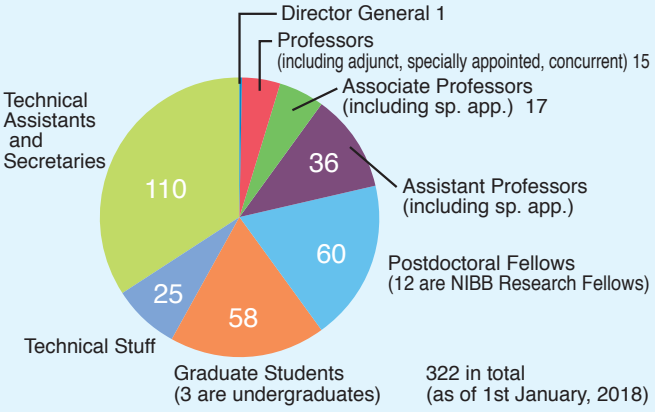
- **Okazaki Administration Office**

Research and Research Support

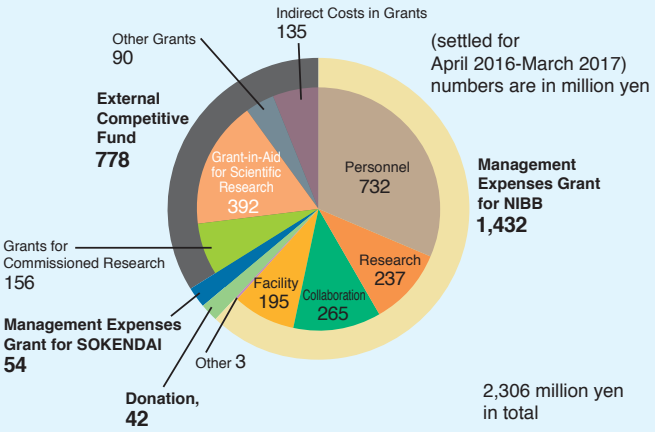
NIBB's research programs are conducted in research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research Facilities and NIBB BioResource Center were launched in 2010 to support research in NIBB and also to act as an intellectual hub to promote collaboration among the researchers of NIBB and other institutions. The NIBB Center of the Inter-University Bio-Backup Project (IBBP Center) was founded in 2012 to prevent loss of invaluable biological resources. Projects for the development of bioresource preservation technology are solicited by the IBBP center from 2013. The NIBB Center for Model Organism Development was founded in 2013 to promote development of new model organisms and research using them. The Technical Division manages the activities of the technical staff and helps to promote the activities of each research unit and to maintain the common resources of both the research support units of NIBB and the research facilities of the Okazaki campus. Center for Radioisotope Facilities are one of the latter and run by the technical staff of NIBB.

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

Members in NIBB



Financial Configuration of NIBB



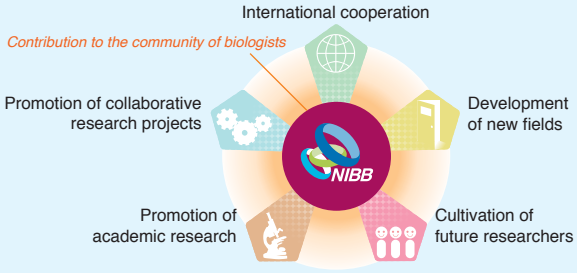
Members of the Advisory Committee for Programming and Management# (as of April, 2017)

Non-NIBB members	AGATA, Kiyokazu*	Professor, Gakushuin University
	HIRAOKA, Yasushi*	Professor, Osaka University
	KOHCHI, Takayuki*	Professor, Kyoto University
	KOHSHIMA, Shiro	Professor, Kyoto University
	KUME, Shoen	Professor, Tokyo Institute of Technology
	NISHITANI, Kazuhiko ##	Professor, Tohoku University
	NOSE, Akinao	Professor, The University of Tokyo
	SASAKI, Hiroyuki*	Professor/ Senior Vice President, Kyushu University
	SUGIMOTO, Asako*	Professor, Tohoku University
	YAMAMOTO, Takashi	Professor, Hiroshima University
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu #	Professor, National Institute for Basic Biology
	HIGASHIJIMA, Shin-ichi	Professor, Okazaki Institute for Integrative Bioscience
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	MINAGAWA, Jun	Professor, National Institute for Basic Biology
	NIIMI, Teruyuki	Professor, National Institute for Basic Biology
	NODA, Masaharu	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, Okazaki Institute for Integrative Bioscience
	UEDA, Takashi*	Professor, National Institute for Basic Biology
	UENO, Naoto	Professor, National Institute for Basic Biology
	YOSHIDA, Shosei	Professor, National Institute for Basic Biology

Chairperson
Vice-Chair
* new member from April 2017

GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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



Promotion of Collaborative Research

Collaborative Research Support

Research activities in collaboration with NIBB's divisions/laboratories using NIBB's facilities are solicited from external researchers. "Individual collaborative research projects" are the basic form of collaboration support which provide external researchers with travel and lodging expenses to visit NIBB's laboratories for collaborative research. "Priority collaborative research projects" are carried out as group research by internal and external researchers to develop pioneering research fields. "Collaborative research projects for model organism/technology development" and "Collaborative research projects for bioresource preservation technology development" are for developing and establishing new model organisms and new research technology. For these projects, research expenses in addition to travel expenses are provided. In 2016 two new projects, Collaborative research projects for integrative genomics and Collaborative research projects for integrative bioimaging, were initiated by reorganizing two former projects to facilitate more integrated use of the NIBB Core Research Facilities and to allow more intensive support through the planning, experimental, data analysis, and publication stages. Travel and lodging expenses are provided for these projects.

NIBB Core Research Facilities

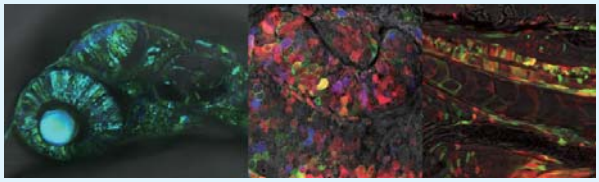
The NIBB Core Research Facilities support research in NIBB and also act as an intellectual hub to promote collaboration between NIBB and other academic institutions. They consist of three facilities that are developing and providing state-of-the-art technologies through functional genomics, bioimaging and bioinformatics (p. 75).

The Functional Genomics Facility maintains a wide array of core research equipment, including next generation DNA sequencers. The facility is dedicated to fostering NIBB's collaborative research by providing these tools as well as expertise. The current focus is supporting functional genomics works that utilize mass spectrometers and DNA sequencers, holding training courses as one of these undertakings (p. 97). The Spectrography and Bioimaging Facility manages research tools, such as confocal microscopes, DSLM and the large spectrograph, and provides technical support and scientific advice to researchers. These two facilities hold specially appointed associate professors, an expert in each field, with a mission to manage each facility as well as conducting his own academic research. The Data Integration and Analysis Facility supports analysis of large-scale biological data, such as genomic sequence data, gene expression data, and imaging data. For this purpose, the facility maintains high-performance computers with large-capacity storage systems.

NIBB BioResource Center

The NIBB BioResource Center supports research using model animals and plants in NIBB and other academic institutions. The center consists of three facilities, the model animal, the model plant, and the cell biology research facilities. The center has equipment, facilities, and staff to maintain model organisms, such as mice, medaka, zebrafish, Japanese morning glory, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, and provides technical support and advice for the appropriate use of these organisms (p. 82).

The center also act as a hub of the National BioResource Project (NBRP) which is a national project for the systematic accumulation, storage, and supply of nationally recognized bio-resources (experimental animals and plants, cells, DNA, and other genetic resources), which are widely used as materials in life science research. To promote this national project, NIBB has been appointed as a center for research on medaka (*Oryzia latipes*), whose usefulness as a vertebrate model was first shown by Japanese researchers. The usability of medaka as a research material in biology has drawn increasing attention since its full genome sequence recently became available. NIBB is also a sub-center for the NBRP's work with Japanese morning glory and zebrafish (p. 85).



An example of a medaka strain in NBRP, Gaudi strain, in which individual cells in the brain and the retina are fluorescently labelled using the Brainbow system.

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

To prevent damage to important biological resources by natural disasters, NIBB established the IBBP Center in 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources under cutting-edge research (p. 86).

Advanced Bioimaging Support (ABiS)

ABiS provides assistance for advanced imaging in the research supported by Grants-in-Aid for Scientific Research. NIBB together with NIPS contribute as core institutes to the ABiS network of domestic partner organizations that own and operate multiple types of advanced specialized imaging equipment (p. 99).

International Cooperation and Outreach

Collaborative Programs with Overseas Institutes

NIBB takes a leading role in the collaborative research programs between the European Molecular Biology Laboratory (EMBL) and the National Institutes of Natural Sciences (NINS) and promotes personal and technological exchange through joint meetings, exchange between researchers and graduate students, and the introduction of experimental equipment.

NIBB formed an agreement with the Temasek Life Sciences Laboratory (TLL) of Singapore and Princeton University to promote joint research projects, collaborative symposia, training courses and student exchange programs. NIBB-Princeton Joint Proteomics Training Course was held in July, 2017 (p. 96).

year	2014	2015	2016	2016
Priority collaborative research projects	1	2	2	2
Collaborative research projects for model organisms and technology development	2	3	2	2
Individual collaborative research projects	87	88	46	51
NIBB workshops	3	6	6	3
Collaborative experiments using the Large Spectrograph	12	10	10	9
Collaborative experiments using the DSLM	10	11		
Bioimage processing and analysis collaborative research projects		14	38*	28*
Collaborative experiments using the next generation DNA sequencer	37	46	59**	62**
Support for NIBB training courses	0	1	0	0
Collaborative research projects for bioresource preservation technology development	10	9	12	12
total	162	190	175	169

*renovated as "Collaborative research projects for integrative bioimaging"
**renovated as "Collaborative research projects for integrative genomics"

NIBB Conference

The NIBB Conferences are international conferences on hot topics in biology organized by NIBB's professors. Since the first conference in 1977 (the year of NIBB's foundation), NIBB Conferences have provided researchers in basic biology with valuable opportunities for international exchange. The 65th conference "Renaissance of *Marchantia polymorpha* –the genome and beyond-" was held in December, 2017 (p. 94).

International Practical Course

With the cooperation of researchers from Japan and abroad the NIBB international practical course is given at a laboratory specifically prepared for its use. The 9th course "Genetics and Imaging of Medaka and Zebrafish" was held jointly with TLL in August, 2016 at NIBB. Graduate students and young researchers from various countries and areas were provided with training in state-of-the-art research techniques. International conferences and courses are managed by the International Cooperation Group of the Research Enhancement Strategy Office (p. 91).

Outreach

NIBB's outreach activities aim to present cutting edge research results to the public via mass media through press releases or directly through the internet. Our triannual open campus event was held in October, 2016 at which we welcomed more than 4,700 local citizens. NIBB also cooperates in the education of undergraduates and younger students through lectures and workshops. Outreach activities are mostly managed by the Public Relations Group of the Research Enhancement Strategy Office (p. 90).

Development of New Fields of Biology

Bioimaging

NIBB aims to maximize the application of modern light microscopes and biophotonic probes for real time visualization of biological phenomena and to develop new imaging techniques. As part of our collaborative work with EMBL, NIBB introduced a DSLM, which is effective for the three-dimensional observation of living organisms, and has developed an improved model using two-photon optics (p. 71). The application of the adaptive optics to microscopy is under way in collaboration with the National Astronomical Observatory of Japan. The Advisory Committee on Bioimaging, comprised of leading researchers in the bioimaging field in Japan, is organized to formulate advice on NIBB's imaging research. The Bioimaging Forum provides an opportunity for researchers and company engineers to frankly discuss practical difficulties and needs regarding imaging. The 11th Forum "Pioneering New Bioimaging by Fusing Optics and Biology" was held in February, 2017 (p. 97). A training course in bioimage analysis was also held in 2017 (p. 98).

Okazaki Biology Conferences

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

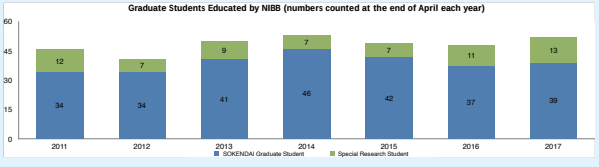
Cultivation of Future Researchers

NIBB constitutes the Department of Basic Biology in the School of Life Science of the SOKENDAI (Graduate University for Advanced Studies). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree. Graduate students enrolled in other universities and institutions can apply to be special research students eligible to conduct research under the supervision of NIBB professors. In both cases above, graduate students can receive financial support from NIBB based on the research assistant (RA) system from the beginning of the five-year course.

Due to the international collaboration with EMBL, graduate students are encouraged to attend PhD student symposia held at EMBL and provided an opportunity to give oral and poster presentations at least once (p. 95).

Students from Japan and abroad can experience NIBB through our Internship Program which gives students an opportunity way to build international connections while experiencing hands on research in a world class research institute (p. 100).

Support for young researchers is managed by the Young Researcher Support Group of the Research Enhancement Strategy Office (p. 92).



Personnel changes in 2017*

Newly assigned in NIBB

Name	Position	Research Unit	Date
NONOMURA, Keiko	Assistant Professor	Division of Embryology	January 16
KONDO, Yohei	Assistant Professor	Division of Quantitative Biology	April 1
KANAZAWA, Takehiko	Assistant Professor	Division of Cellular Dynamics	April 1
FUJITA, Hironori	Assistant Professor	Division of Symbiotic Systems	April 1
MATSUDA, Takashi	NIBB Research Fellow	Division of Molecular Neurobiology	April 1
SAKAI, Yusuke	NIBB Research Fellow	Division of Morphogenesis	April 1
GOTO, Yuhei	NIBB Research Fellow	Division of Quantitative Biology	May 1
YOSHIDA, Saiko	NIBB Research Fellow	Division of Evolutionary Biology	June 1
MANO, Shoji	Associate Professor	Research Enhancement Strategy Office	November 1
ANSAI, Satoshi	Assistant Professor	Laboratory of Bioresources	November 1
ARATA, Masaki	NIBB Research Fellow	Division of Embryology	December 1

Newly affiliated in other universities and institutes

Name	New Affiliation	Position	Date
TAKEHANA, Yusuke	Nagahama Institute of Bio-Science and Technology	Associate Professor	April 1
TAKEDA, Naoya	Kwansei Gakuin University	Associate Professor	April 1
OHDE, Takahiro	Kyoto University	Assistant Professor	June 1
YOSHIDA, Saiko	Max Planck Institute for Plant Breeding Research	Marie Curie Fellow	August 1

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

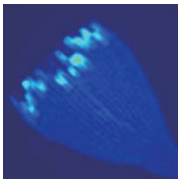
Awardees in 2017

Name	Position	Award
MATSUDA, Takashi	NIBB Research Fellow	22nd Nagakura Research Incentive Award
SHIINA, Nobuyuki	Associate Professor	Astellas Award for the Best Biomedical Research
NORIZUKI, Takuya	Graduate Student	Taiwan-Japan Plant Biology 2017 Outstanding Poster Award

Selected Press Releases from NIBB in 2017

■ 27 January

Discovery of a plant stem cell inducing factor having a similar structure to an animal stem cell inducing factor



The moss *Physcomitrella patens* Cold-Shock Domain Protein 1 (PpCSP1) regulates reprogramming of differentiated leaf cells to chloronema apical stem cells and shares conserved domains with the induced pluripotent stem cell factor Lin28 in mammals. PpCSP1 accumulates in the reprogramming cells and is maintained throughout the reprogramming process and in the resultant stem cells. Genetic manipulation experiments demonstrated a positive role of PpCSP1 in reprogramming, which is similar to the function of mammalian Lin28 (see p. 45).

Li, C., Sako, Y., Imai, A., Nishiyama, T., Thompson, K., Kubo, M., Hiwatashi, Y., Kabeya, Y., Karlson, D., Wu, S.-H., Ishikawa, M., Murata, T., Benfey, P.N., Sato, Y., Tamada, Y., and Hasebe, M. (2017). A Lin28 homolog reprograms differentiated cells to stem cells in the moss *Physcomitrella patens*. Nat. Commun. 8, 14242.

■ 7 February

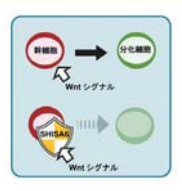
Genome of the pitcher plant Cephalotus reveals genetic changes associated with carnivory

To investigate the molecular bases of carnivory, our research group sequenced the genome of the heterophyllous pitcher plant *Cephalotus follicularis*, in which they succeeded in regulating the developmental switch between carnivorous and non-carnivorous leaves. Transcriptome comparison of the two leaf types and gene repertoire analysis identified genetic changes associated with prey attraction, capture, digestion and nutrient absorption. These results imply constraints on the available routes to evolve plant carnivory (see p. 45).

Fukushima, K., Fang, X., et al. (2017). Genome of the pitcher plant *Cephalotus* reveals genetic changes associated with carnivory. Nat. Ecol. Evol. 1, 0059.

■ 10 February

A mechanism regulating self-renewal and differentiation of sperm stem cells -how stem cells are protected from differentiation-promoting signals

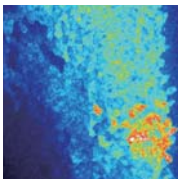


In the seminiferous tubules of mouse testes, a population of GFRα1-positive spermatogonia harbors stem cell functionality. The research group showed that activation of Wnt/β-catenin signaling promotes sperm differentiation and reduces the GFRα1^{+} cell pool. They further discovered that SHISA6, characterized as a cell-autonomous Wnt inhibitor, confers GFRα1^{+} cells resistance to the Wnt/β-catenin signaling. The difference in the effect of Wnt signaling caused by the presence or absence of SHISA6 can produce both self-renewing stem cells or differentiating cells in the uniform tissue environment (see p. 35).

Tokue, M., Ikami, K., Mizuno, S., Takagi, C., Miyagi, A., Takada, R., Noda, C., Kitadate, Y., Hara, K., Mizuguchi, H., Sato, T., Taketo, M.M., Sugiyama, F., Ogawa, T., Kobayashi, S., Ueno, N., Takahashi, S., Takeda, S., and Yoshida, S. SHISA6 confers resistance to differentiation-promoting Wnt/β-catenin signaling in mouse spermatogenic stem cells. Stem Cell Reports, 8, 561-575, 2017.

■ 7 March

Fluctuation in the concentration of calcium ions contributes to neural tube shape formation

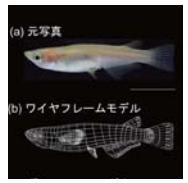


Our research group showed that during the process of neural tube formation a transient increase in the concentration of calcium ions in cells causes morphological changes and is essential for neural tube formation. The group observed the cell population during neural tube formation in *Xenopus laevis* embryos using the fluorescent protein GECO, an intracellular Ca^{2+} concentration indicator. They found that the pattern of the fluctuation in intracellular calcium ion concentration in the cell population is complex. Local and transient rises in intracellular calcium ion concentrations have been found to cause cell deformation and contribute to the formation of the neural tube (see p. 26).

Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Campbell, RE., and Ueno, N. (2017). Distinct intracellular Ca^{2+} dynamics regulate apical constriction and differentially contribute to neural tube closure. Development 144, 1307-1316.

■ 12 April

Three-dimensional computer graphic animation for studying social approach behavior in medaka fish

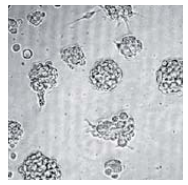


Our research group studied social approach behavior in medaka fish using three-dimensional computer graphic (3DCG) animations. 3DCG virtual fish lacking any combinations of four features (color, shape, locomotion, and body motion) of real medaka fish were created and presented to live fish using a computer display. Medaka fish presented with virtual fish with four normal features spent a long time in proximity to the display, whereas time spent near the display was decreased with other virtual fish lacking any of the four features. The results suggested that the naturalness of visual cues contributes to the induction of social approach behavior (see p. 44).

Nakayasu, T., Yasugi, M., Shiraishi, S., Uchida, S., and Watanabe, E. (2017). Three-dimensional computer graphic animations for studying social approach behaviour in medaka fish: Effects of systematic manipulation of morphological and motion cues. PLoS ONE 12, e0175059.

■ 17 July

Identification of PTPRZ as a drug target for cancer stem cells in glioblastoma



Glioblastoma is the most malignant brain tumor with high mortality. Cancer stem cells are thought to be crucial for tumor initiation and its recurrence after the standard therapy with radiation and temozolomide (TMZ) treatment. An NIBB research group showed that protein tyrosine phosphatase receptor type Z (PTPRZ) is requisite for the maintenance of stem cell properties and tumorigenicity in glioblastoma cells. The research group discovered NAZ2329, an allosteric inhibitor of PTPRZ, in collaboration with a pharmaceutical company. NAZ2329 efficiently suppressed stem cell-like properties of glioblastoma cells in culture, and tumor growth in C6 glioblastoma xenografts. Notably, tumor growth was inhibited more effectively by co-treatment with NAZ2329 and TMZ than by the individual treatments. Thus, pharmacological inhibition of PTPRZ activity is a promising strategy for the treatment of malignant gliomas (see p. 38).

Fujikawa, A., Sugawara, H., Tanaka, T., Matsumoto, M., Kuboyama, K., Suzuki, R., Tanga, N., Ogata, A., Masumura, M., and Noda, M. (2017). Targeting PTPRZ inhibits stem cell-like properties and tumorigenicity in glioblastoma cells. Sci. Rep. 7, 5609.

■ 4 September

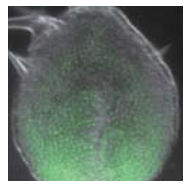
Discovery of dynamic seasonal changes in color perception ~The small fish “medaka” shows large differences in color perception in summer and winter~

Our research group found that color perception of Medaka, a small fish inhabiting rice fields and streams, varies greatly according to seasonal changes. Medaka in the summer conditions were strongly attracted to virtual medaka which showed nuptial coloration, but medaka in the winter conditions were not. It further became clear that expression of opsins, the proteins responsible for the first step in vision, and genes related to signal transduction pathways downstream of opsins are markedly reduced in winter conditions, whereas expression of these genes rises by shifting to summer conditions. This phenomenon of seasonal change in color perception may be a phenomenon widely preserved in various animals (see p. 67).

Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M., Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Comm. 8, 412.

■ 6 September

Building a morphogen gradient by simple diffusion in a growing plant leaf

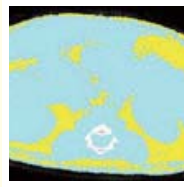


Our research group showed that a transcriptional co-activator ANGUSTIFOLIA3 (AN3) forms a signaling gradient along the leaf proximal-to-distal axis to determine cell-proliferation domain. They demonstrated that pure diffusion in a growing tissue is sufficient to explain the AN3 gradient formation. This work provides evidence that the diffusion-based model of morphogen is viable in developmental patterning of multicellular organisms (see p. 29).

Kawade, K., Tanimoto, H., Horiguchi, G., and Tsukaya, H. (2017). Spatially different tissue-scale diffusivity shapes ANGUSTIFOlIA3 gradient in growing leaves. Biophys. J. 113, 1109-1120.

■ 14 September

Identification of PTPRJ as a regulator of leptin signaling and obesity

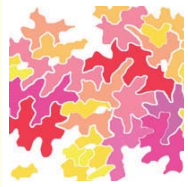


Leptin, an adipocyte-derived hormone, strongly inhibits food intake by acting on the hypothalamus, a part of the mid brain, and plays a crucial role in the body weight control. Circulating leptin levels are closely correlated with the degree of adiposity. An NIBB research group demonstrated that protein tyrosine phosphatase receptor type J (PTPRJ) negatively regulates leptin signaling by inhibiting the activation of leptin receptor-associated JAK2, through the dephosphorylation of Y813 and Y868 in JAK2 autophosphorylation sites. Diet-induced obesity and the leptin treatment both up-regulated PTPRJ expression in the hypothalamus, while the overexpression of PTPRJ induced leptin resistance. Thus, the induction of PTPRJ is a factor contributing to the development of leptin resistance, and the inhibition of PTPRJ may be a potential strategy for improving obesity (see p. 38).

Shintani, T., Higashi, S., Suzuki, R., Takeuchi, Y., Ikaga, R., Yamazaki, T., Kobayashi, K., and Noda, M. (2017). PTPRJ inhibits leptin signaling, and induction of PTPRJ in the hypothalamus is a cause of the development of leptin resistance. Sci. Rep. 7, 11627.

■ 20 September

Rolling dice for cell size specification in plant leaf epidermis

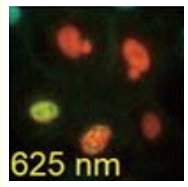


Because size distribution often shows a characteristically skewed pattern in a tissue, there may be some stochastic option for determining cell size. Our research group discovered that endoreduplication, which promotes cellular enlargement in the epidermal tissue of *Arabidopsis thaliana*, occurs randomly as a Poisson process throughout cellular maturation. These results link the probabilistic property of endoreduplication dynamics to cell size distribution, providing a theoretical background to explain how size heterogeneity is established within a leaf (see p. 73).

Kawade, K., and Tsukaya, H. (2017). Probing the stochastic property of endoreduplication in cell size determination of *Arabidopsis thaliana* leaf epidermal tissue. PLoS ONE 12, e0185050.

■ 24 October

Efficient synthesis of a photosynthetic pigment in mammalian cells for Optogenetics

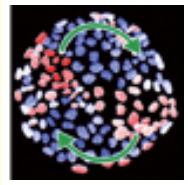


The “Optogenetic” method of controlling the position of proteins in cells using light is becoming widely employed as an important method to understand intracellular signal transduction. The Phytochrome B (PhyB)-PIF system made way to the use of red light / near infrared light, which have low toxicity and good tissue penetrance. However in the case of animal cells the cyanobacterial photosynthetic pigment phycocyanobilin (PCB) must be added from outside, and this step greatly hindered the use of the PhyB-PIF system. NIBB researchers have now succeeded in synthesizing PCB in animal cells directly by introducing four genes encoding cyanobacterial enzymes related to PCB synthesis. In addition, the group succeeded in increasing the amount of PCB synthesis through disruption of the gene that encodes an enzyme called biliverdin reductase A, which is involved in the metabolism of PCB (see p. 15).

Uda, Y., Goto, Y., Oda, S., Kohchi, T., Matsuda, M., and Aoki, K. An efficient synthesis of phycocyanobilin in mammalian cells for optogenetic control of cell signaling. Proc. Natl. Acad. Sci. USA 2017 Oct 24. doi:10.1073/pnas.1707190114

■ 7 November

Discovery of a mechanism for determining the direction of collective cell migration



A group at NIBB have found that when the activity of a molecule called ERK MAP kinase is propagated to neighboring cells, the cells migrate in the opposite direction of ERK propagation. In cells cultured on a circular micro-patterned glass base dish, a spontaneous rotational wave of ERK activity was observed and the cells rotated in the opposite direction. Furthermore, by artificially creating waves of ERK activity by optogenetic techniques, the research group succeeded in making cells move collectively (see p. 15).

Aoki, K., Kondo, Y., Naoki, H., Hiratsuka, T., Itoh, R.E., and Matsuda, M. Propagating wave of ERK activation orients collective cell migration. Develop. Cell 2017 Nov 6. doi:10.1016/j.devcel.2017.10.016

■ 21 November

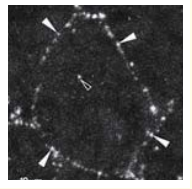
mRNA localization regulatory factor RNG105/caprin1 is essential for long-term memory formation

Researchers at NIBB have revealed that the function of RNG105 (aka Caprin1) is essential for the formation of long-term memory. Mice who have lost RNG105 in the cerebrum and hippocampus can form short-term memories of several minutes, but the long-term memory, developed over several days to one week, does not form in these mice. They also found that RNG105-regulated mRNA localization to dendrites is involved in the correct placement of AMPA receptors in synapses, which are known to be important for memory retention (see p. 23).

Nakayama, K., Ohashi, R., Shinoda, Y., Yamazaki, M., Abe, M., Fujikawa, A., Shigenobu, S., Futatsugi, A., Noda, M., Mikoshiba, K., Furuichi, T., Sakimura, K., and Shiina, N. (2017). RNG105/caprin1, an RNA granule protein for dendritic mRNA localization, is essential for long-term memory formation. eLife 6, e29677.

■ 7 December

Two types of heparan sulfate clusters may constitute a cellular platform for the distribution and signaling of Wnt8, a morphogen molecule

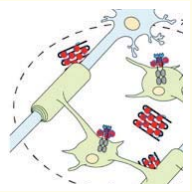


Wnt proteins direct embryonic patterning, but the regulatory basis of their distribution and signal reception remain unclear. Our research group showed that endogenous Wnt8 protein is distributed in a graded manner in *Xenopus* embryo and accumulated on the cell surface in a punctate manner in association with "N-sulfo-rich heparan sulfate (HS)," not with "N-acetyl-rich HS". These two types of HS clusters may constitute a cellular platform for the distribution and signaling of Wnt8 (see p. 29).

Mii, Y., Yamamoto, T., Takada, R., Mizumoto, S., Matsuyama, M., Yamada, S., Takada, S., and Taira, M. (2017). Roles of two types of heparan sulfate clusters in Wnt distribution and signalling in *Xenopus*. Nat. Comm. 8, 1973.

■ 8 December

Protamine neutralizes CSPG-mediated inhibition of oligodendrocyte differentiation



Remyelination is a critical repair process in demyelinating diseases such as multiple sclerosis (MS). Damage to the central nervous system (CNS) results in a glial reaction, leading to the formation of a glial scar enriched with chondroitin sulfate proteoglycans (CSPGs). CSPGs also accumulate as constituents of demyelinating plaques in MS lesions, which inhibit the migration and differentiation of oligodendrocyte precursor cells and remyelination. An NIBB research group found that a polycationic peptide, protamine (PRM) neutralized the inhibitory activity of aggrecan, a representative extracellular matrix CSPG molecule, and that PRM functioned as an inhibitory ligand mimetic of protein tyrosine phosphatase receptor type Z (PTPRZ), a membrane-spanning CSPG predominantly expressed in oligodendrocyte precursor cells. The intranasal administration of PRM accelerated myelination in the developing mouse brain, and its intracerebro-ventricular administration stimulated remyelination after cuprizone-induced demyelination. These results indicate that PRM has CSPG-neutralizing activity, thereby promoting oligodendrocyte differentiation under developmental and morbid conditions (see p. 38).

Kuboyama, K., Tanga, N., Suzuki, R., Fujikawa, A., and Noda, M. (2017). Protamine neutralizes chondroitin sulfate proteoglycan-mediated inhibition of oligodendrocyte differentiation. PLoS ONE 12, e0189164.

DIVISION OF CELLULAR DYNAMICS

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Membrane traffic between single membrane-bounded organelles plays integral roles in various cell activities in eukaryotic cells. Recent comparative genomics has indicated that membrane trafficking pathways are diversified among eukaryotic lineages, which is associated with lineage-specific acquisition of new trafficking pathways and secondary loss of preexisting trafficking routes. Our long-term goal is unraveling how plants have acquired their unique membrane trafficking systems during evolution, which will be achieved by comparative analyses using the model plant *Arabidopsis thaliana* and a model of liverworts, *Marchantia polymorpha*. We also aim to elucidate detailed molecular mechanisms and physiological functions of membrane trafficking in higher-ordered plant functions.

I. Diversification of membrane trafficking pathways associated with acquisition of novel machinery components

Although the basic framework of membrane trafficking is well conserved among eukaryotic lineages, recent comparative genomics has suggested that each lineage has acquired unique membrane trafficking pathways during evolution. RAB GTPases and SNARE proteins are evolutionarily conserved key regulators acting in tethering and/or fusion of membrane vesicles with target membranes. It has been proposed that lineage-specific diversification of these key factors is tightly associated with acquisition of lineage-specific membrane trafficking pathways, whose molecular basis remains unknown.

1-1 Characterization of RAB and SNARE proteins in the liverwort, *Marchantia polymorpha*

For information on the diversification of membrane trafficking pathways during land plant evolution, we systematically identified RAB GTPases and SNARE proteins in *Marchantia polymorpha*. Comparison of organization of these protein families with other plant lineages, followed by their functional analyses in *M. polymorpha*, indicated that diversification of membrane trafficking pathways in land plants has been achieved by 1) acquisition of novel machinery components, 2) relocating conserved machinery components to distinct trafficking events, and 3) secondary loss of conserved machinery components, during evolution.

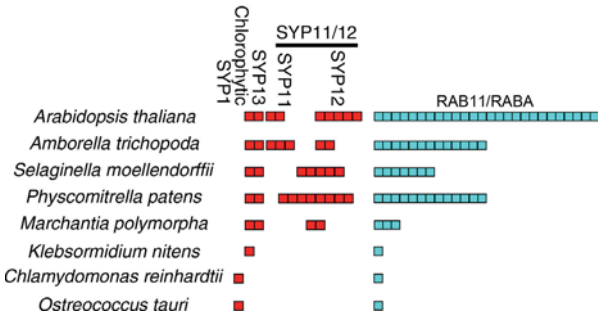

Figure 1. *SYP1* and *RAB11* genes in the genomes of green plants. Genes are indicated as individual red (for *SYP1*) or cyan (for *RAB11*) boxes. Numbers of these genes increased during land plant evolution, suggesting secretion-related functions have been diversified in land plants.

1-2 Analysis of the liverwort-specific organelle, oil body

Through analyses of SNARE members in *M. polymorpha*, we found that a member of the SYP1 group is localized to the membrane of an organelle specific to liverworts, the oil body (Figure 2), whose origin and mechanisms of biogenesis remain unclear. We are currently analyzing the molecular function of the SYP1 member, as well as characterizing membrane trafficking pathways responsible for oil body biogenesis. We have succeeded in isolating several mutants defective in the function and morphogenesis of the oil body through a forward-genetic approach. Analyses of a mutant with an increased number of oil bodies identified a transcription factor regulating oil body biogenesis, which activates transcription of genes responsible for secondary metabolite biosynthesis and putative transporters, as well as the SYP1 member.

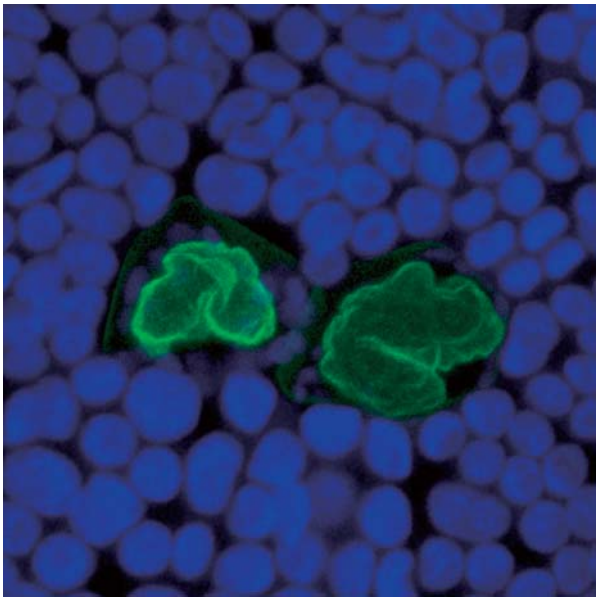

Figure 2. Oil bodies in a thallus of *M.* polymorpha, which are visualized by the YFP-tagged SYP1 member (green). Autofluorescence from chlorophyll is also shown (blue).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

1-3 Mechanisms and dynamics of vacuolar transport

The vacuole is the largest organelle in plant cells, and occupies over 90% of mature plant cells. The vacuole fulfills various functions in plant physiology and development, which include protein degradation, protein storage, and regulation of turgor pressure. To fulfill these vacuolar functions, a wide variety of vacuolar proteins and other components must be correctly transported to the vacuole, which is mediated by membrane trafficking. To understand molecular mechanisms of vacuolar transport in plants, we analyzed molecular functions of RAB5 and RAB7, and found that these proteins act sequentially in the vacuolar transport pathway in Arabidopsis cells. Furthermore, we also found that two additional vacuolar transport pathways, the RAB5-dependent but RAB7-independent pathway and the AP-3-dependent pathway operate in vacuolar transport in Arabidopsis. We are now exploring details of molecular mechanisms of these vacuolar transport pathways, especially focusing our interest on the RAB5-dependent but RAB7-independent pathway, because this trafficking pathway has not been described in non-plant systems. A tethering complex, CORVET, is known to act as an effector of Vps21/RAB5 to regulate endosomal transport in yeast and animal cells. CORVET subunits are also conserved in Arabidopsis, but molecular function of CORVET remained unclear. We found that VPS3, one of the CORVET subunits, acts in the RAB5-dependent and RAB7-independent vacuolar transport pathway in Arabidopsis (Figure 3). Furthermore, we also found that another tethering complex sharing the core complex with CORVET, the HOPS complex, regulates a different trafficking event from CORVET. Our results further indicated that different fusion machineries comprising distinct R-SNARE proteins are involved in CORVET- and HOPS-mediated trafficking pathways. These findings demonstrated that the plant vacuolar transport system has been diverged from vacuolar/lysosomal transport systems in non-plant systems.

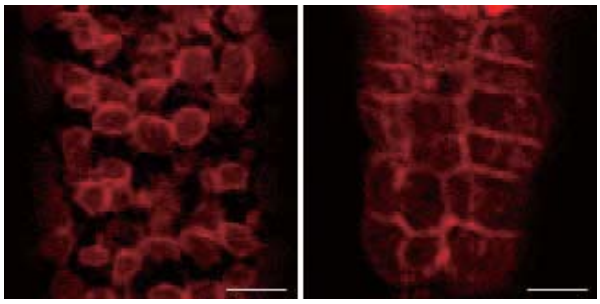

Figure 3. Subcellular localization of mRFP-SYP22 in WT-like and *vps3* embryos. SYP22 is one of the cargo molecules of the RAB5-dependent and RAB7-independent vacuolar transport pathway. mRFP-tagged SYP22 localized to the vacuolar membrane in WT-like embryo cells (left), whereas it mislocalized to the plasma membrane in the *vps3* mutant embryo cells (right). Bars = 10 μm.

We also conducted detailed analyses of vacuolar SNARE proteins. Defective functions in vacuolar SNAREs affect both vacuolar transport and vacuolar morphology. The *sgr3-1* (*shoot gravitropism3*) mutant was isolated as a mutant defective in shoot gravitropism, which resulted from a point mutation in SYP22/VAM3, one of the SNARE proteins residing on the vacuole and acting in vacuolar transport. Intriguingly, *sgr3-1* exhibits abnormal vacuolar morphology, although vacuolar transport is not markedly affected in this mutant. Therefore *sgr3-1* should be a good tool for dissecting functions of the vacuolar SNARE. We are exploring vacuolar dynamics regulated by SYP22 by analyzing the effect of the *sgr3-1* mutation in a detailed manner. Co-immunoprecipitation and MS analyses indicated that the *sgr3-1* mutation specifically stabilizes a SNARE complex comprising VAMP71, SYP22, and αSNAP. We also found that these molecules and HOPS components accumulate on the vacuolar membrane in *sgr3-1*. These results highlighted a unique characteristic of the *sgr3-1* mutation, which will provide valuable information to better understand mechanisms of SNARE-mediated membrane fusion.

II. Significance of membrane trafficking in higher-ordered plant functions

2-1 Analyses of functions of the plant-specific RAB GTPase ARA6 in stress responses in *Arabidopsis thaliana*

ARA6 is a plant-unique RAB GTPase, whose close homologs are only found in green plant lineages. To elucidate why only plants harbor the ARA6 members, we analyzed functional significance of ARA6 in biotic and abiotic stress responses. We found that ARA6 is recruited to the extrahaustorial membranes formed by the fungal pathogen causing powdery mildew and the oomycete causing downy mildew, suggesting modulation of host membrane trafficking by pathogenic microbes (Inada *et al*., 2016). We also found that overexpression of constitutive active ARA6 repressed the full proliferation of powdery mildew fungi (Inada et al., 2017).

2-2 Membrane trafficking in plant gametogenesis

Gametogenesis in plants also involves membrane trafficking-mediated processes. We are now analyzing molecular mechanisms of gametogenesis in Arabidopsis and *M. polymorpha*, especially focusing our interests on secretory and degradative trafficking pathways during male gamete formation.

Cytokinesis in land plants is achieved by re-direction of the secretory pathway, and KNOLLE/SYP111 and KEULE/SEC11 play important roles in membrane fusion at the forming cell plate in somatic cells of Arabidopsis. Conversely, any deleterious effects on gametogenesis have been reported for mutations in these genes thus far. We found that other SYP1 and SEC1 members are highly expressed during male gametogenesis. The analyses of these proteins during male gametogenesis are currently underway.

Distinct from seed plants, basal land plants including *M. polymorpha* utilize sperm as the male gamete in sexual reproduction. We visualized the process of sperm formation, especially spermiogenesis, using fluorescently-tagged organelle markers (Figure 4). The majority of the endomembranous organelles such as the Golgi apparatus was removed from the maturing cells, and the plasma membrane was also reorganized during spermiogenesis. A TEM analysis also indicated that the number of degradative organelles such as the multivesicular endosomes, vacuoles, and autopha-

gosomes, was transiently increased during this process. To reveal the molecular mechanisms of organelle degradation and cytoplasm removal, we are now analyzing the contribution of autophagy. The autophagy-defective mutations resulted in defective morphogenesis and motility of sperm, indicating crucial functions of autophagy during spermiogenesis in *M. polymorpha*.

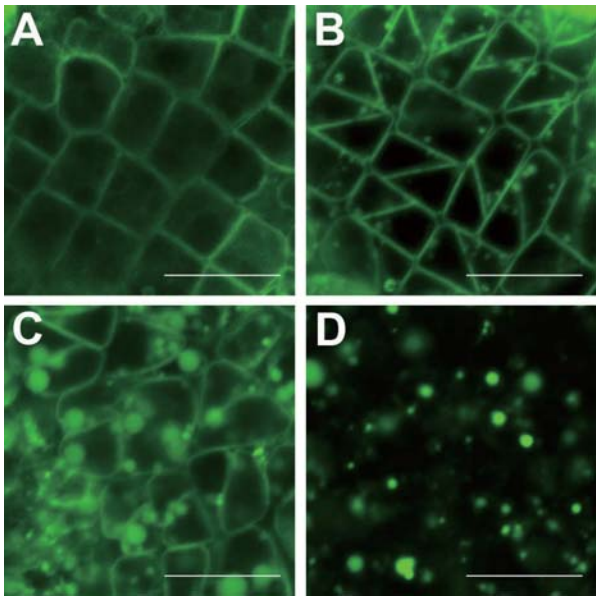

Figure 4. Reorganization of the plasma membrane during spermatogenesis in *M. polymorpha*. Spermatogenesis proceeds from A to D. A plasma membrane-resident protein visualized with Citrine is targeted to intracellular spherical structures, and completely removed from the plasma membrane of mature spermatids. Bars = 10 μm. (Adopted from Minamino et al., 2017)

Publication List:

〔Original papers〕

- Akita, K., Kobayashi, M., Sato, M., Kutsuna, N., Ueda, T., Toyooka, K., Nagata, N., Hasezawa, S., and Higaki, T. (2017). Accumulation of fluorescent proteins derived from a trans-Golgi cisternal membrane marker and paramural bodies in interdigitated apoplastic space in Arabidopsis leaf epidermis. Protoplasma *254*, 367-377.
- Bowman, J.L., Kohchi, T., Yamato, K.T., Jenkins, J., Shu, S., Ishizaki, K., Yamaoka, S., Nishihama, R., Nakamura, Y., Berger, F., et al. (2017). Insights into land plant evolution garnered from the Marchantia polymorpha genome. Cell *171*, 287-304.
- Cui, Y., Zhao, Q., Xie, H.T.,Wong, W.S., Gao, C., Ding, Y., Tan, Y., Ueda, T., Zhang, Y., and Jiang, L. (2017). MON1/CCZ1-mediated Rab7 activation regulates tapetal programmed cell death and pollen development in Arabidopsis. Plant Physiol. *173*, 206-218.
- Inada, N., Ebine, K., Ito, E., Nakano, A., and Ueda, T. (2017). Constitutive activation of plant-specific RAB5 GTPase confers increased resistance against adapted powdery mildew fungus. Plant Biotech. *34*, 89-95.
- Ito, Y., Toyooka, K., Fujimoto, M., Ueda, T., Uemura, T., and Nakano A. (2017). The trans-Golgi network and the Golgi stacks behave independently during regeneration after Brefeldin A treatment in tobacco BY-2 cells. Plant Cell Physiol. *58*, 811-821.
- Matsui, H., Nomura, Y., Egusa, M., Hamada, T., Hyon, G.S., Kaminaka, H., Watanabe, Y., Ueda,, T., Trujillo, M., Shirasu, K., and Nakagami, H. (2017). The GYF domain protein PSIG1 dampens the induction of cell death during plant-pathogen interactions. PLoS Genet. *13*, e1007037.
- Minamino, N., Kanazawa, T., Nishihama, R., Yamato, T.K., Ishizaki, K., Kohchi, T., Nakano, A., and Ueda, T. (2017). Dynamic reorganization of the endomembrane system during spermatogenesis in Marchantia polymorpha. J. Plant Res. *130*, 433-441.
- Ung, H., Karia, P., Ebine, K., Ueda, T., Yoshioka, K., and Moeder, W. (2017). Triphosphate tunnel metalloenzyme function in senescence highlights a biological diversification of this protein superfamily. Plant Physiol. *175*, 473-485.

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

- Sánchez-Rodríguez, C., Shi, Y., Kesten, C., Zhang, D., Sancho-Andrés, G., Ivakov, A., Lampugnani, E.R., Sklodowski, K., Fujimoto, M., Nakano, A., Bacic, A., Wallace, I.S., Ueda, T., van Damme, D., Zhou, Y., and Persson, S. The cellulose synthases are cargo of the TPLATE adaptor complex. Mol. Plant 2017 Dec 5.

〔Review article〕

- Kanazawa, T., and Ueda, T. (2017). Exocytic trafficking pathways in plants: why and how they are redirected. New Phytol. *215*, 952-957.

DIVISION OF QUANTITATIVE BIOLOGY

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Assistant Professor:	*KONDO, Yohei*
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	UDA, Youichi
	MARYU, Gembu
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Living cells act as input-output (I/O) units, in which environment and/or internal states are recognized on the cell surface and processed within a cell, leading to the adaptive response to these changes. This cellular information processing is mainly controlled by intracellular signal transduction, which is comprised of a series of chemical reactions, most commonly protein phosphorylation. Importantly, dysregulation of the signal transduction by gene mutation results in pathological diseases such as cancer.

The intracellular signaling pathway has been extensively studied over the last few decades, and most of its proteins and regulations have been identified, causing the perceived increase in the pathway's complexity. Computer-assisted simulation is one of the most promising approaches for the understanding of the signal transduction pathway as a system. Indeed, chemical and physical reactions constituting the signal transduction can be described by a set of ordinary differential equations, and solved numerically by computers. A number of simulation models of signaling pathways have, in fact, been reported to date. However, most of the kinetic parameters utilized for the simulation models were not measured experimentally, but rather were assumed by fitting the experimental data with the simulation model or simply determined arbitrarily. Consequently, there are substantial differences in the kinetic parameters between these studies, thereby making it difficult to evaluate these simulation models quantitatively.

To address these issues, we currently focus on the development of research tools enabling us to (1) visualize, (2) quantify, and (3) manipulate the intracellular signaling pathways with fluorescence imaging techniques and computational approaches.

I. Visualization of cell signaling

With the advent of fluorescent proteins, it has been possible to visualize kinetic reactions at the single cell level. Förster (or fluorescence) resonance energy transfer (FRET) is a non-radiative process, in which excitation energy of donor fluorophore is transferred to a nearby acceptor fluorophore. Taking advantage of the principles, FRET-based biosensors allowed us to detect kinase activity of PKA, ERK, Akt, JNK, PKC, and S6K in a living cell with high temporal and spatial resolution (Komatsu N, Mol. Biol. Cell, 2011) (Figure 1).

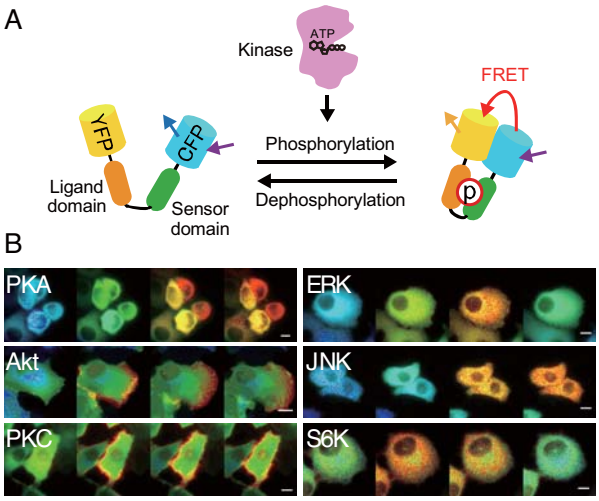


Figure 1. Visualization of kinase activity in living cells. (A) Schematic representation of intramolecular FRET biosensor of kinases. (B) PKA, ERK, Akt, JNK, PKC and S6K activities by FRET imaging. HeLa cells expressing FRET biosensors for the indicated kinases were stimulated with each ligand.

By using a FRET biosensor, we revealed that intercellular propagating waves of ERK activation determined the direction of the collective cell migration (Figure 2). Cell migration is a fundamental process in many physiological and pathological contexts. Collective cell migration refers to a movement of cell groups with physical and functional cell-to-cell connections, and is inherently involved in the processes of embryonic development, wound healing, and cancer invasion. However, it remains unclear how cells in a group organize physical and chemical clues into synchronized directionality and migration to facilitate coordinated collective migration. We visualized ERK activity in collectively migrating Mardin-Darby canine kidney (MDCK) cells by an ERK FRET biosensor, EKAREV, and found the

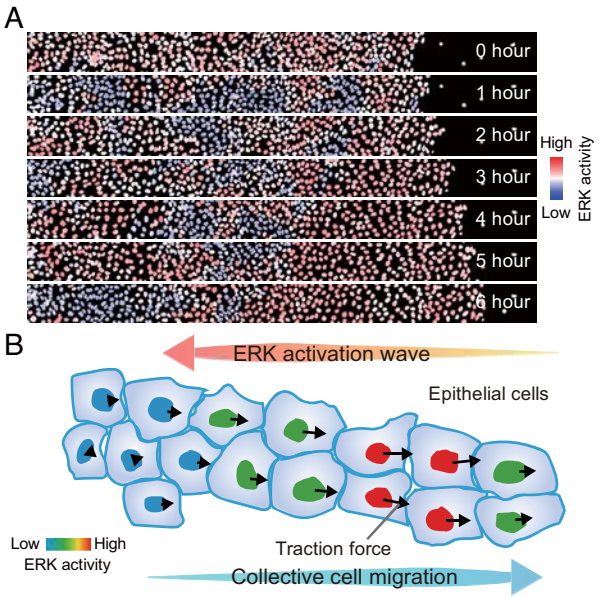


Figure 2. ERK activation wave and collective cell migration. (A) Propagating waves of ERK activation in wound healing of MDCK cells. (B) Schematic representation of collective cell migration driven by ERK activation propagation.

intercellular propagating waves of ERK activation (Figure 2A). Interestingly, MDCK cells collectively migrated against the direction of the ERK activation wave. The inhibition of ERK activation propagation suppressed collective cell migration. The ERK activation wave spatiotemporally controlled actomyosin contraction and cell density. Furthermore, an artificially-generated ERK activation wave by optogenetics reproduces induction of the collective cell migration. These data provide new mechanistic insights into how cells sense the direction of collective cell migration (Aoki K, Dev Cell, 2017).

Although FRET biosensors enable us to monitor cell signaling events, FRET imaging is not suited for multiplexed imaging, because these biosensors are authentically composed of two different fluorophores in many cases. To circumvent this limitation, we are currently developing biosensors based on the principle of the kinase translocation reporter (KTR) system; the reporter is translocated from nucleus to cytoplasm when it is phosphorylated by its target kinase (Figure 3). This approach yields several kinase reporters, e.g., ERK, Akt, p38, and JNK, and will provide clues as to how cells emerge all-or-none and irreversible cellular events such as cell cycle progression and apoptosis (Maryu G, Cell Strut, Funct, 2016; Miura H, bioRxiv, 2017) (Figure 3).

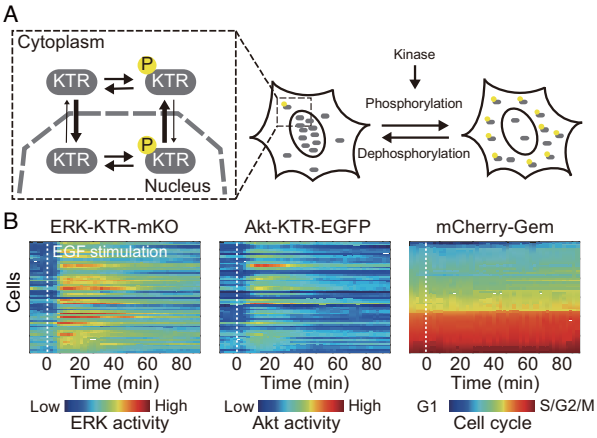


Figure 3. Multiplexed imaging of ERK and Akt activities and cell cycle state. (A) Schematic representation of the principle of KTR system. (B) Shown here are heat maps of ERK activity (left), Akt activity (middle), and cell cycle (right) obtained by KTR systems.

II. Quantification of cell signaling

Classically, kinetic parameters such as protein concentration and dissociation constant, Kd, have been measured by *in vitro* biochemical analyses. However, some kinetic parameters might significantly differ between *in vitro* and *in vivo*. For instance, the Kd values measured *in vivo* were higher than the *in vitro* Kd values by an order of 1 or 2 (Sadaie W, MCB, 2014). Therefore, it is critical to measure kinetic parameters in living cells.

To this end, we launched a research project of quantitative cell cycle modeling in fission yeast *Schizosaccharomyces pombe* and mammalian cultured cells. *S. Pombe* offers many advantages for studying the cell cycle; this organism shares most of the genes used for the cell cycle by mammalian cells and shows efficient labeling of endogenous genes with conventional knock-in techniques. We established several *S. Pombe* lines expressing cyclin and cyclin-dependent kinase fused with GFP and RFP, respectively, and quantified these expression levels during the process of the cell cycle by live cell imaging. In mammalian cells, we developed an efficient knock-in system with CRISPR/Cas9-mediated genome editing techniques (Figure 4), and combined with fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) to quantitatively determine the protein concentration and the dissociation constant of endogenous protein (Komatsubara A, bioRxiv, 2017)(Figure 4).

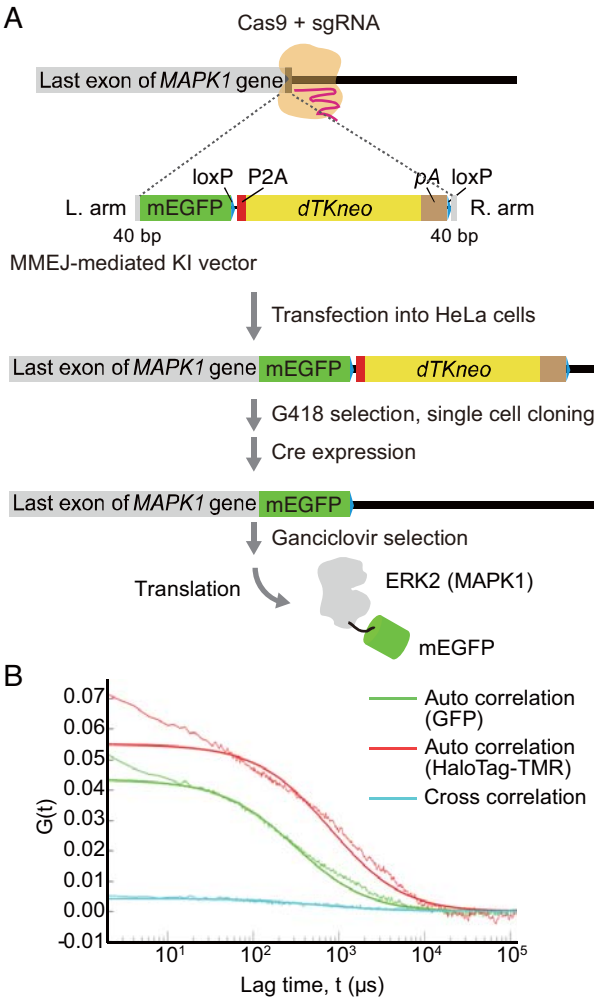


Figure 4. Quantification of protein concentration and dissociation constant of endogenous proteins. (A) Schematic illustration of gene knock-in with an MMEJ-mediated KI vector at MAPK1 locus. (B) Auto- and cross-correlation functions of ERK2-mEGFP and RSK2-HaloTag. *mEGFP* and *HaloTag* genes were knocked-in at the site of 3' *ERK2* and *RSK2* genes, respectively.

III. Manipulation of cell signaling

Artificial manipulation of biochemical networks would be useful for quantitative understanding of biological systems. Multiple methods have been suggested for controlling signal transduction, including light-induced dimerization (LID) and chemically induced dimerization (CID) systems. Among them the LID system has strong advantages in terms of temporal and spatial manipulations. The photo-responsive

proteins derived from fungi, cyanobacteria and plants, and modified fluorescent proteins are used in this system.

We focus on the phytochrome B (PhyB)-PIF LID system: Upon red-light illumination, PhyB binds to PIF, and the PhyB-PIF complex dissociates from each other by infra-red light exposure (Figure 5A). The reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system, because either association or dissociation is regulated by light in other LID systems. One drawback is that covalent attachment of a chromophore, e.g. phycocyanobilin (PCB), is required for the light-responsive function of PhyB-PIF. To overcome this issue, we developed a method for synthesis of phycocyanobilin in mammalian cells by introduction of the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into mitochondria (Uda Y, PNAS, 2017) (Figure 5B). The amount of synthesized PCB was comparable to externally-delivered PCB. We could demonstrate light-induced ERK activation with the PhyB-PIF LID system combined with endogenously synthesized PCB, showing the artificial oscillation of ERK activation (Figure 5C and 5D). The genetically encoded system of PCB synthesis will provide a potential advantage for establishing transgenic animals that stably synthesize PCB endogenously, thereby enabling the optogenetic manipulation of cell signaling in deeper tissues without injecting PCB.

Publication List:

〔Original papers〕

- Aoki, K., Kondo, Y., Naoki, H., Hiratsuka, T., Itoh, R.E., and Matsuda, M. (2017). Propagating wave of ERK activation orients collective cell migration. Dev. Cell *43*, 305–317 e5.
- Uda, Y., Goto, Y., Oda, S., Kohchi, T., Matsuda, M., and Aoki, K. (2017). Efficient synthesis of phycocyanobilin in mammalian cells for optogenetic control of cell signaling. Proc. Natl. Acad. Sci. USA *114*, 11962–11967.

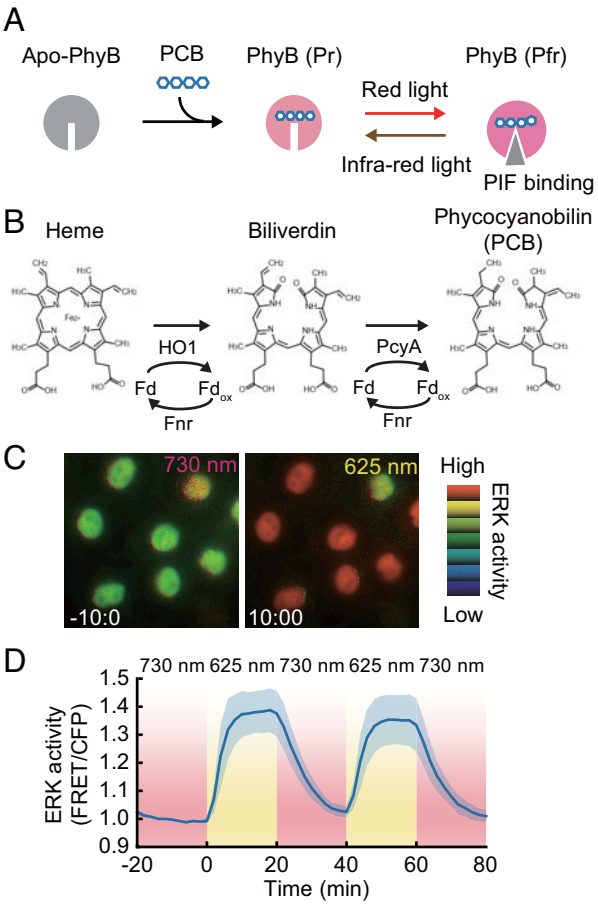
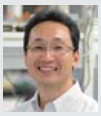


Figure 5. PhyB-PIF light-inducible dimerization (LID) system with PhyB-PIF and its application to the manipulation of cell signaling. (A) Apo-PhyB covalently attaches its chromophore, which is phycocyanobilin (PCB) to produce holo-PhyB. There are two holo-PhyB forms, PhyB (Pr) and PhyB (Pfr), which change over in manner dependent on the chromophore status. Only PhyB (Pfr) associates with PIF. (B) The metabolic pathway of phytochrome chromophores. (C) Light-induced activation of ERK with PhyB-PIF LID system. Representative images of ERK activity at the nucleus in HeLa cells. (D) The average ERK activity (normalized FRET/CFP ratio) is plotted as a function of time with the standard deviation.

DIVISION OF CHROMATIN REGULATION



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The stable inheritance of gene expression or repression state is essential to cellular differentiation and development, and an epigenetic cellular-memory system derived from higher-order chromatin structure plays a fundamental role in this process. The assembly of higher-order chromatin structure has been linked to the covalent modifications of histone tails, chromatin-associated proteins, and non-coding RNAs. The exact means by which such chromatin-based epigenetic information is established and faithfully maintained across cell divisions and throughout development, however, remains incompletely understood. To try to gain a better understanding of the molecular mechanisms underlying chromatin-based epigenetic phenomena, our lab uses fission yeast, *Schizosaccharomyces pombe*, and ciliate *Tetrahymena* as model organisms for studying the molecular mechanisms of higher-order chromatin assembly. We are also attempting to determine the cellular functions of chromatin modifying factors in developing a better understanding of complicated epigenetic phenomena in higher eukaryotic cells.

I. Establishment and maintenance of higher-order chromatin structure

In eukaryotic cells, the assembly of higher-order chromatin structure, known as heterochromatin, plays an important role in diverse chromosomal processes. We have previously shown that the specific methyl modification on lysine 9 of histone H3 (H3K9me) and the binding of the chromodomain (CD) proteins such as HP1 to methylated histones are essential to the assembly of higher-order chromatin structure. SUV39H is the major H3K9-specific methyltransferase that targets the pericentric regions and is crucial for assembling silent heterochromatin. SUV39H recognizes trimethylated H3K9me3 via its CD, and enriched H3K9me3 allows SUV39H to target specific chromosomal regions. However, the detailed targeting mechanisms, especially for naïve chromatin without preexisting H3K9me3, are poorly understood. We showed that Suv39h1's CD (Suv39h1-CD) binds nucleic acids, and that this binding is important for its function in heterochromatin assembly. Suv39h1-CD had higher binding affinity for single-stranded RNA than double-stranded DNA, and its ability to bind nucleic acids was independent of its H3K9me3 recognition. Suv39h1 bound major satellite RNAs *in vivo*, and mutational studies demonstrated that both the nucleic acid–binding and H3K9me-binding activities of Suv39h1-CD were crucial for its pericentric heterochromatin assembly (Figure 1). These results suggest that chromatin-bound RNAs contribute to creating SUV39H's target specificity.

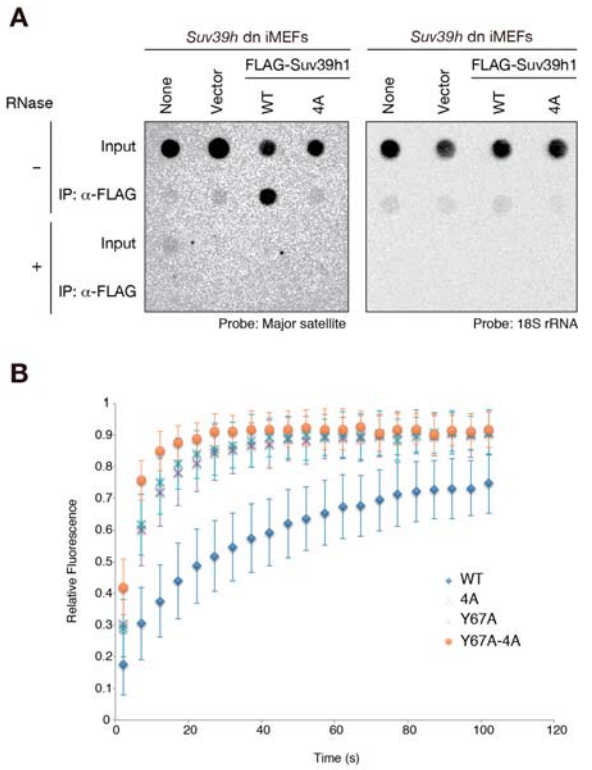

Figure 1. Suv39h1-CD's nucleic acid binding is required for its interaction with major satellite RNAs and chromatin. (A) Dot-blot analysis of immunoprecipitated RNAs. RNAs associated with WT or mutant (4A) Suv39h1 in Suv39h dn iMEFs were precipitated with the anti-FLAG M2 antibody and subjected to dot-blot analysis using a labeled probe for major satellite repeats (left) and 18S rRNA (right). (B) FRAP analysis of WT or mutant GFP-Suv39h1 in suv39h dn iMEFs.

II. Regulation of chromodomain proteins in assembling higher-order chromatin structure

2-1 Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding

HP1 is an evolutionarily conserved chromosomal protein that plays a crucial role in heterochromatin-mediated gene silencing. We previously showed that mammalian HP1α is constitutively phosphorylated at its N-terminal serine residues by casein kinase II (CK2), and that this phosphorylation enhances HP1α's binding specificity for nucleosomes containing H3K9me3. Although the presence of additional HP1α phosphorylation during mitosis was reported more than a decade ago, its biological significance remains largely elusive. Here we found that mitosis-specific HP1α phosphorylation affected HP1α's ability to bind chromatin. Using biochemical and mutational analyses, we showed that HP1α's mitotic phosphorylation was located in its hinge region and was reversibly regulated by Aurora B kinase and two serine/threonine phosphatases. In addition, chromatin fractionation and electrophoretic mobility shift assays revealed that hinge region-phosphorylated HP1α was preferentially dissoci-

ated from mitotic chromatin and exhibited a reduced DNA-binding activity. Although HP1α's mitotic behavior was previously linked to H3 serine 10 phosphorylation, which blocks the binding of HP1α's chromodomain (CD) to H3K9me3, our findings suggest that mitotic phosphorylation in HP1α's hinge region also contributes to changes in HP1α's association with mitotic chromatin.

2-2 Phosphorylation of CBX2 controls its nucleosome-binding specificity

Chromobox 2 (CBX2), a component of polycomb repressive complex 1 (PRC1), binds lysine 27–methylated histone H3 (H3K27me3) via its chromodomain (CD) and plays a critical role in repressing developmentally regulated genes. The phosphorylation of CBX2 has been described in several studies, but the biological implications of this modification remain largely elusive. We showed that CBX2's phosphorylation plays an important role in its nucleosome binding. CBX2 is stably phosphorylated *in vivo*, and domain analysis showed that residues in CBX2's serine-rich (SR) region are the predominant phosphorylation sites. The serine residues in an SR region followed by an acidic-residue (AR) cluster coincide with the consensus target of casein kinase II (CK2), and CK2 efficiently phosphorylated the SR region *in vitro*. A nucleosome pull-down assay revealed that CK2-phosphorylated CBX2 had a high specificity for H3K27me3-modified nucleosomes (Figure 2). An electrophoretic mobility-shift assay showed that CK2-mediated phosphorylation diminished CBX2's AT-hook–associated DNA-binding activity. Mutant CBX2 lacking the SR region or its neighboring AR cluster failed to repress the transcription of p21, a gene targeted by PRC1. These results suggest that CBX2's phosphorylation is critical for its transcriptional repression of target genes.

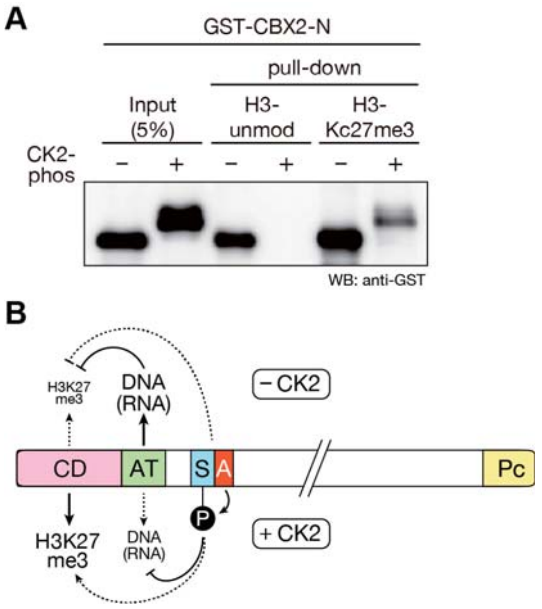

Figure 2. CK2-mediated phosphorylation of CBX2-N increases its binding specificity for H3K27me3 nucleosomes. (A) A representative nucleosome pull-down assay using synthesized nucleosomes containing unmodified H3 (H3-unmod) or H3 with a K27me3 analog (H3Kc27me3). (B) Proposed model for the role of CK2-mediated phosphorylation in the chromatin binding of CBX2.

III. Proteomic approach to identify chromatin-bound RNA-binding proteins

Recent advances in methodologies and technologies led to systematic identification of more than 1,000 RNA-binding proteins (RBPs) in mouse and human cells. Some of these RBPs are known to locate in the nucleus. Interestingly, previous studies suggest that RNAs together with their RBP partners do not only contribute to transcriptional regulation of individual genes, but also play a part in three-dimensional organization of gene loci within the nucleus and/or in regulation of the inner-nuclear structures. However, currently available information is rather fragmentary and molecular mechanisms by which RNA-RBP complexes mediate local and global effects remain largely unknown. Moreover, it is unclear if RNA-RBP interactions participate in other chromatin-based biological processes. In order to grasp the whole picture of physical or functional interactions between RNAs and RBPs in the nucleus, it is essential to identify which RNA-RBP complexes are actually bound to chromatin/nuclear substructures. We have developed a new proteomics-based method to systematically identify such RBPs as potential chromatin-/nuclear matrix-associated RBPs and successfully identified previously uncharacterized RBPs (Figure 3). We will verify their RNA-mediated chromatin interaction and investigate their roles in chromatin organization and transcriptional regulation.

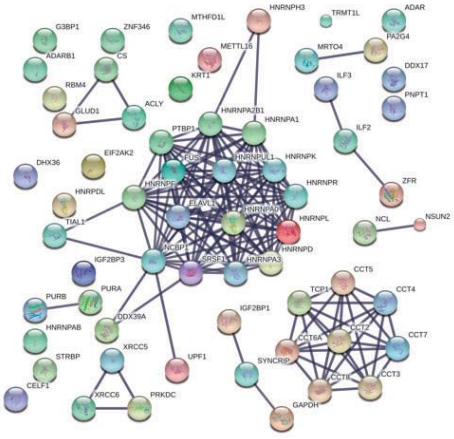

Figure 3. Network analysis of the identified RNase-sensitive nuclear proteins.

IV. Dynamic chromatin reorganization during sexual reproduction of *Tetrahymena*

Tetrahymena, a unicellular protozoan, shows nuclear dimorphism. It contains the transcriptionally silent, germline micronucleus (MIC) and the transcriptionally active, somatic macronucleus (MAC) in the single cell. During sexual reproduction, the newly developed MAC removes thousands of internal eliminated sequences (IESs), which are derived from transposable elements, from the genome. The IES elimination requires RNA interference (RNAi)-related pathway and posttranslational histone modifications. The methylation of histone H3 at lysine 9 and lysine 27 catalyzed by Ezl1p act as binding sites for chromodomain proteins such as Pdd1p to form heterochromatin-like structures. Although previous studies identified more than 20 factors that are linked to

heterochromatin formation and/or DNA elimination, their precise roles in chromatin reorganization remain poorly understood. We have analyzed the cellular localization of potential heterochromatin factors during MAC development and identified 3 novel proteins as heterochromatin components. We have also conducted an ectopic DNA elimination assay and demonstrated that 10 proteins are sufficient for inducing DNA elimination (Figure 4). We will further characterize these heterochromatin proteins and define their roles in chromosome reorganization and IES elimination.

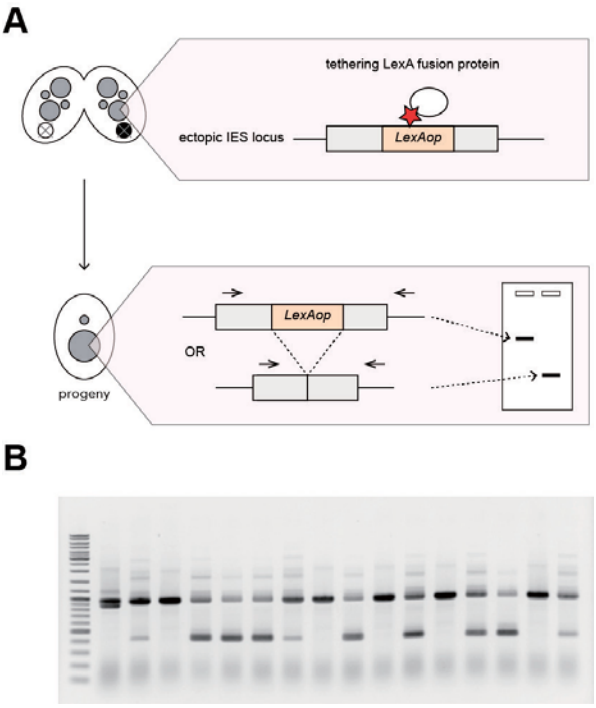

Figure 4. Identification of heterochromatin proteins sufficient to induce DNA elimination. (A) Schematics of the tethering assay. (B) A representative result of the tethering assay using one of heterochromatin proteins.

Publication List:

〔Original papers〕

- Eustache, S., Créchet, J.-B., Bouceba, T., Nakayama, J., Tanaka, M., Suzuki, M., Woisard, A., Tuffery, P., Baouz, S., and Hountondji, C. (2017). A functional role for the monomethylated Gln-51 and Lys-53 residues of the 49GGQTK53 motif of eL42 from human 80S ribosomes. Open Biochem. J. *11*, 8-26.
- Kawaguchi, T., Machida, S., Kurumizaka, H., Tagami, H., and Nakayama, J. (2017). Phosphorylation of CBX2 controls its nucleosome-binding specificity. J. Biochem. *162*, 343-355.
- Mutazono, M., Morita, M., Tsukahara, C., Chinen, M., Nishioka, S., Yumikake, T., Dohke, K., Sakamoto, M., Ideue, T., Nakayama, J., Ishii, K., and Tani, T. (2017). The intron in centromeric noncoding RNA facilitates RNAi-mediated formation of heterochromatin. PLoS Genet. *13*, e1006606.
- Shirai, A., Kawaguchi, T., Shimojo, H., Muramatsu, D., Ishida-Yonetani, M., Nishimura, Y., Kimura, H., Nakayama, J., and Shinkai, Y. (2017). Impact of nucleic acid and methylated H3K9 binding activities of Suv39h1 on its heterochromatin assembly. Elife *6*, e25317.
- Zafar, F., Okita, A.K., Onaka, A.T., Su, J., Katahira, Y., Nakayama, J., Takahashi, T.S., Nasukata, H., and Nakagawa, T. (2017). Regulation of mitotic recombination between DNA repeats in centromeres. Nucleic Acids Res. *45*, 11222-11235.

LABORATORY OF CELL RESPONSES

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Cells sense the environment around them, for example the amount of nutrients and hormones, as well as the temperature and pressure, and decide what kind of activities to undertake using this information. Germ cells, which produce sperm and eggs, begin halving their number of chromosomes during a special kind of cell division called meiosis, in response to the ambient conditions. In our laboratory, we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis (Figure 1), to research the mechanism by which cells switch from mitosis, the kind of cell division that divides cells equally to create two identical cells, to meiosis, which is essential for bringing forth genetically diverse progeny.

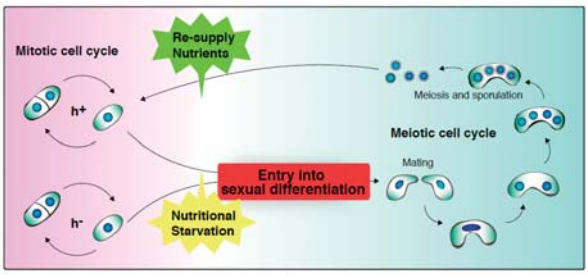

Figure 1. Life cycle of the fission yeast *S. pombe*. *S. pombe* cells proliferate by mitotic growth under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. Resulting diploid zygotes undergo meiosis and produce spores.

I. Signaling pathways that regulate the onset of sexual differentiation

We have been trying to elucidate how *S. pombe* cells switch their mode of cell cycle from mitotic to meiotic. We focus on a highly conserved kinase, namely Target of rapamycin (TOR) kinase, which plays key roles in the recognition of nutrition and the onset of sexual differentiation in *S. pombe*. TOR kinase forms two types of complexes, namely TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential to suppress sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for onset of sexual differentiation under nitrogen starvation (Figure 2).

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich medium at the restrictive temperature. To gain insights into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions. In most mutants identified, TORC1 activity is downregulated and the mutated genes are involved in tRNA expression or modification. We are currently characterizing these mutants.

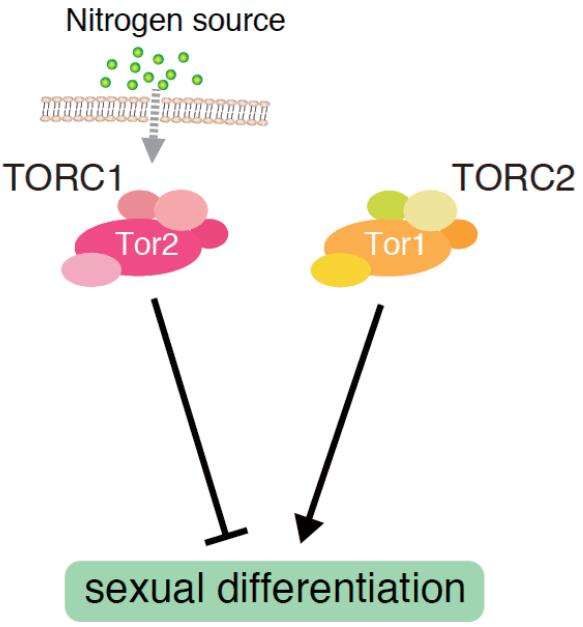

Figure 2. The two TOR complex pathways in *S. pombe*. TORC1, containing Tor2, and TORC2, containing Tor1, regulate sexual differentiation oppositely. TORC1 suppresses sexual differentiation in the presence of ample nitrogen.

II. The molecular mechanisms that establish the meiosis-specific gene expression profile

Expression of hundreds of genes are upregulated during meiosis. We have shown that specific control of the stability of meiotic transcripts, which is orchestrated by the interplay between RNA-binding proteins and a long non-coding RNA, contributes to the meiosis-specific gene expression in the fission yeast *S. pombe*. Understanding precise mechanisms of this control will shed light on the regulation of timely gene expression during meiosis.

A YTH-family RNA-binding protein Mmi1 plays a crucial role in the selective elimination system of meiosis-specific transcripts during the mitotic cell cycle. Mmi1 recognizes a region termed DSR (Determinant of Selective Removal) in meiotic transcripts, which is enriched with repeats of hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machinery. Mmi1 also induces formation of facultative heterochromatin at a subset of its target genes.

During meiosis, a meiosis-specific nuclear body, called Mei2 dot, blocks the Mmi1-mediated elimination system. The Mei2 dot is composed of the RNA-binding protein Mei2 and a long non-coding RNA species termed meiRNA. Mei2 physically interacts with meiRNA and forms the dot structure at the chromosomal *sme2* locus, which encodes meiRNA. The Mei2 dot lures Mmi1 through numerous copies of the DSR motif on meiRNA and inhibits its function, so that meiotic transcripts harboring DSR are stably expressed (Figure 3).

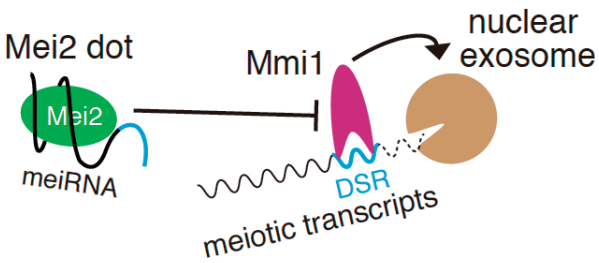

Figure 3. Selective elimination of meiosis-specific transcripts by the Mmi1/DSR system. Mmi1 binds to DSR in meiotic transcripts and induces their degradation by the nuclear exosome during the mitotic cell cycle. In meiotic cells, the Mei2 dot, composed of Mei2 and meiRNA, sequesters and inhibits Mmi1, so that DSR-harboring meiotic transcripts escape from Mmi1-mediated selective elimination.

We have shown that Mmi1 regulates the termination of transcription of its target genes. Mmi1-mediated termination of an upstream non-coding RNA ensures the expression of downstream genes, one of which encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) essential for the initiation of sexual differentiation. We have also demonstrated that Mmi1 prevents untimely expression of meiotic proteins by tethering their mRNAs to nuclear foci. Multilayered suppression of meiotic genes by Mmi1 is vital for mitotic growth.

Publication List:

〔Original paper〕

● Touat-Todeschini, L., Shichino, Y., Dangin, M., Thierry-Mieg, N., Gliquin, B., Hiriart, E., Sachidanandam, R., Lambert, E., Brettschneider, J., Reuter, M., Kadlec, J., Pillai R., Yamashita, A., Yamamoto., M., and Verdel, A. (2017). Selective termination of lncRNA transcription promotes epigenetic silencing and cell differentiation. EMBO J. *36*, 2626-2641.

〔Review article〕

● Otsubo, Y., Yamamoto, M., and Yamashita, A. (2017). TORC1-dependent phosphorylation targets in fission yeast. Biomolecules *7*, 50.

LABORATORY OF NEURONAL CELL BIOLOGY

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Technical Assistant: *MATSUDA, Chisato*

The transport of specific mRNAs and local control of translation in neuronal dendrites represent an important gene expression system that provides dendritic protein synthesis at just the right time and place. It is widely accepted that this system controls the location at which neurites will connect to each other, thereby forming neural networks. Our main interest is to understand the mechanisms and roles of mRNA transport and local translation in neuronal dendrites.

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

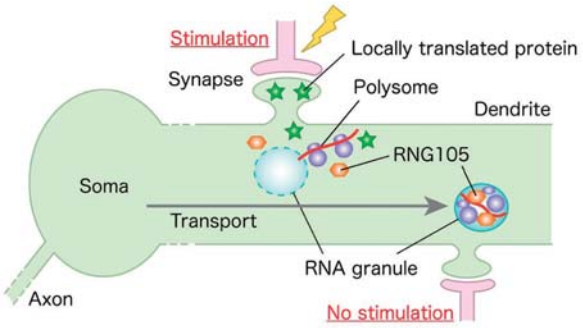

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. RNG105 is essential for long-term memory formation

We previously identified RNA granule protein 105 (RNG105, also known as Caprin1), an RNA-binding protein, as a major component of RNA granules. RNG105 promotes the assembly of RNA granules and is responsible for the transport of its binding mRNAs in cultured cells.

To understand the role of RNG105 in learning and memory, we subjected RNG105 conditional knockout (cKO) mice, which lacked the *Rng105* gene in the brain region, to contextual fear conditioning tests. One of the tests was a passive avoidance test, in which an apparatus consisting of light and dark chambers was used (Figure 2). Mice received foot shock in the dark chamber. After that, if they remember the context, i.e., fear in the dark chamber, they would not enter the dark chamber and would stay in the light chamber. At 5 min after the foot shock, RNG105 cKO mice did not enter the dark chamber any more than control mice. However, at 1 day after the foot shock, they spent significantly longer time in the dark chamber than control mice. Furthermore, at 1 week after the foot shock, they spent as long a time as before the foot shock (Figure 2). These results indicated that not short-term memory, but long-term memory was markedly impaired by RNG105 cKO.

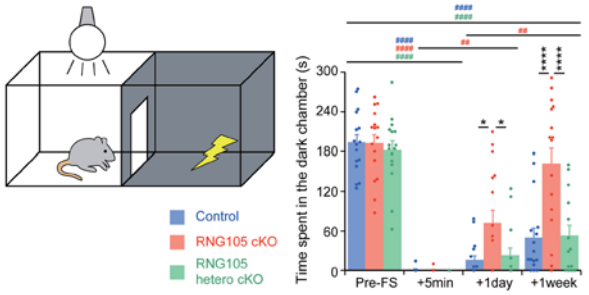

Figure 2. Contextual fear conditioning test. Time spent in the dark chamber before foot shock (Pre-FS) and at 5 min, 1 day and 1 week after the fear conditioning.

Another test was a contextual freezing test. In this test, mice received foot shock in a single chamber and their freezing responses were measured in the same chamber at 5 days after the foot shock. RNG105 cKO mice showed less freezing behavior than control mice, which indicated that RNG105 cKO mice did not well remember the place they learned to fear. These results supported the notion that RNG105 is essential for the formation of long-term memory.

II. RNG105 deficiency impairs AMPA receptor scaling on dendrites

To investigate the mechanism of how RNG105 cKO affects long-term memory, we measured electrophysiological responses (fEPSP) of neurons to synaptic stimulation in the hippocampus, the essential brain region for memory formation. fEPSP in RNG105 cKO mice was reduced by half compared to control mice both in the steady state and after synaptic long-term potentiation (LTP). This suggested that AMPA receptors (AMPARs), key factors for the synaptic transmission, were downregulated by RNG105 deficiency.

AMPAR downregulation by RNG105 deficiency was further suggested by RNA-seq analysis as follows. We comprehensively identified mRNAs whose dendritic localization was reduced in hippocampal neurons from RNG105 cKO mice. Subsequent gene ontology enrichment analysis revealed that a major category of the reduced mRNAs was "Regulation of Arf protein signal transduction", which included GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) of small G protein Arf. Arf is known to regulate membrane trafficking, which includes the regulation of AMPAR surface expression on dendrites. These results suggested that cell surface expres-

sion of AMPARs on dendrites was affected by RNG105 deficiency.

We then quantified AMPARs expressed on the surface of dendrites in cultured neurons by immunostaining (Figure 3). Comparison between control and RNG105-deficient neurons revealed that the number of surface AMPARs on dendrites was significantly reduced by RNG105 deficiency. Furthermore, the scaling mechanism for AMPARs on the surface of dendrites was impaired in RNG105-deficient neurons: although normal neurons increase the surface number of AMPARs upon neuronal activity deprivation in order to maintain the basal activity, RNG105-deficient neurons did not increase the surface number of AMPARs even when their activity was blocked by tetrodotoxin (TTX) and APV (Figure 3). This impaired scaling of AMPARs is considered to underlie the reduced synaptic responses in the basal state, which may also attenuate synaptic responses in the LTP state.

Taken together, our study demonstrated that an element of RNA granules, RNG105, is essential for long-term memory formation. Our study further revealed RNG105-dependent dendritic localization of mRNAs as an underlying mechanism for AMPAR-dependent synaptic strength and long-term memory formation.

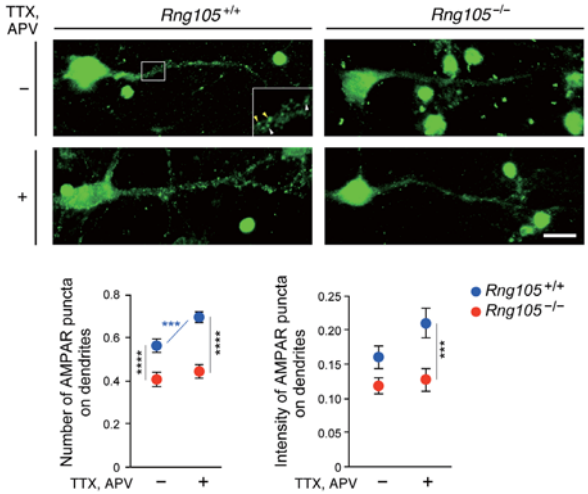

Figure 3. RNG105 deficiency impairs AMPAR scaling on dendrites. Primary cultured neurons from the cerebral cortex of control (*Rng105*$^{+/+}$) and RNG105 knockout (*Rng105*$^{-/-}$) littermates were immunostained for surface-expressed AMPAR subunit GluR1. Arrowheads in the inset indicate AMPAR puncta. The number of AMPAR puncta on dendrites is less in *Rng105*$^{-/-}$ neurons than in *Rng105*$^{+/+}$ neurons. Although the number of AMPAR puncta was increased by TTX and APV treatment in *Rng105*$^{+/+}$ neurons, it was not increased in *Rng105*$^{-/-}$ neurons. Bar, 10 μm.

Publication List:

〔Original paper〕

● Nakayama, K.*, Ohashi, R.*, Shinoda, Y., Yamazaki, M., Abe, M., Fujikawa, A., Shigenobu, S., Futatsugi, A., Noda, M., Mikoshiba, K., Furuichi, T., Sakimura, K., and Shiina, N. (2017). RNG105/caprin1, an RNA granule protein for dendritic mRNA localization, is essential for long-term memory formation. eLife *6*, e29677. (*: equal contribution)

LABORATORY OF STEM CELL BIOLOGY

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DNA is constantly damaged from both endogenous and exogenous sources. One of the most important challenges for all living organisms is to prevent genome instability that can lead to malfunction of a cell. Our group is interested in the strategies through which cells protect themselves from alterations in the genome. To date, much information is gained from various model organisms and tissue culture cells, and we are beginning to learn that the choice of genome-maintenance strategies taken by a cell depends on the cell type, cell cycle- and developmental stages. Our focus is on the genome maintenance mechanisms of the embryonic stem cells, and their roles during differentiation and reprogramming processes.

I. Self-renewal of Embryonic Stem Cells and Their Genome-Maintenance Mechanisms

Embryonic stem (ES) cells are derived from the blastocyst stage of embryonic development, and are capable of differentiating into all cell types that compose our body (i.e., ES cells are "pluripotent"). Pluripotent cells exist only transiently and are lost as development proceeds. On the other hand, ES cells are capable of proliferating indefinitely when given an appropriate culturing condition. Curiously, ES cells proliferate with truncated gap phases while S (DNA replication) and M (mitosis) phases take as much time as other cell types. ES cells also appear to lack some of the mechanisms that ensure genome integrity (i.e., checkpoint mechanisms), the significance of which remains a mystery.

To date, studies on cell cycle regulation in ES cells have not been straightforward compared to that of other cell types, as many commonly used cell-synchronization protocols are ineffective for ES cells. We have now established several protocols to synchronize ES cells (Tsubouchi et al., Cell, 2013; unpublished), which allowed us to investigate specific stages of the ES cell cycle. So far, we have found that DNA replication is regulated differently in ES cells, such that DNA replication of the whole genome is more accurate in ES cells. We are currently aiming to address how such differences are interlinked with pluripotency by carrying out side-by-side analyses between ES cells and differentiated populations.

II. Genome Instability during Nuclear Reprogramming

In order to gain a deeper understanding of the relationship between the choice of genome maintenance mechanisms and pluripotency, we are investigating the behavior of factors involved in genome maintenance mechanisms during nuclear reprogramming towards pluripotency.

Specifically, we take advantage of the cell-to-cell fusion approach, in which a target cell is fused to a pluripotent stem cell to induce pluripotency within a target nucleus. The cell fusion system is a simple, versatile way to induce reprogramming towards another lineage, not limited to pluripotency. Using this system, the first sign of reprogramming can be detected from within a few hours to one day after fusion, allowing us to monitor the initial events of reprogramming after induction.

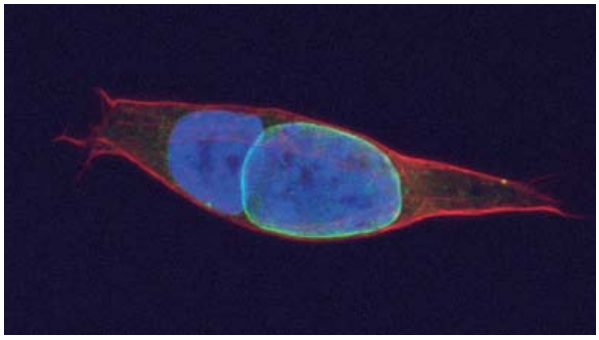

Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus (unpublished).

Using this system, we previously found that DNA synthesis is an important event for successful reprogramming (Tsubouchi et al., Cell, 2013). Recent reports indicate that reprogramming may cause genetic instabilities, some of which are thought to arise as DNA replication errors. To investigate the nature of such errors and how they are linked to reprogramming-specific events, we are in the process of setting up a system to isolate and track a single fused cell through live-imaging (Figure 1).

III. Future Perspective

While the fundamental mechanisms that maintain genome integrity have been widely studied using various models, the danger a cell might face when altering their cellular identity (through differentiation, reprogramming etc.) is unknown. Recent studies of cancer genome sequencing repeatedly identified mutations in the factors that govern cellular identities, leading us to hypothesize that cells may experience genome instability when their identity is unstable. Our goal is to uncover the nature of such genetic instability and to gain a comprehensive understanding of the mechanisms that maintain genome integrity.

Publication List:

〔Original paper〕

● Argunhan, B., Leung, W.-K., Afshar, N., Terentyev, Y., Subramanian, V., Murayama, Y., Hochwagen, A., Iwasaki, H., Tsubouchi, T.*, and Tsubouchi, H.* (2017). Fundamental cell cycle kinases collaborate to ensure timely destruction of the synaptonemal complex during meiosis. EMBO J. *36*, 2488-2509. * corresponding authors

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The complex morphogenesis of organisms is achieved by dynamic rearrangements of tissues during embryogenesis, in which change in cellular morphology as well as orchestrated cell movements are involved. For cells to know how they should change their shape and where they should move, information called “cell polarity” is essential. How then is the cell polarity established within cells? Is it intrinsically formed within the cells or triggered by extracellular cues? Furthermore, little is known as to how coordinated and complex cell movements are controlled in time and space. We attempt to understand the mechanisms underlying these events using several model animals, including frogs, fish, mice and ascidians, taking physical parameters such as force in consideration, in addition to conventional molecular and cellular biology.

I. Cell behaviors during gastrulation

During embryogenesis, the arrangement of multicellular tissue is dramatically changed to establish properly shaped embryos. These movements of groups of cells are often highly organized and collective. Investigating the mechanism of collective cell migration is therefore essential for understanding embryogenesis. *Xenopus* leading edge mesoderm (LEM) is one of the suitable models for studying this morphogenetic movement, because of easy micromanipulation of tissue excised from the embryo. During *Xenopus* gastrulation, LEM moves into the blastocoel ahead of the axial mesoderm, which forms the notochord and muscles in the future. In our previous studies, we have shown that LEM generates the driving force of mesodermal migration, and measured the physical value of this force with the explant. To address how each single LEM cell generates the force for collective migration in the explant, we have introduced Traction Force Microscopy (TFM) for *Xenopus* LEM explant migration and found that traction force was generated by the cells, which were located at the anterior part of explants. Particularly in the first few rows of cells of LEM explants, frequent intracellular Ca^{2+} transients were observed. We have also been able to demonstrate that this spatiotemporally controlled Ca^{2+} dynamics is essential for the anterior-directed tissue migration through the regulation of the asymmetric lamellipodia formation.

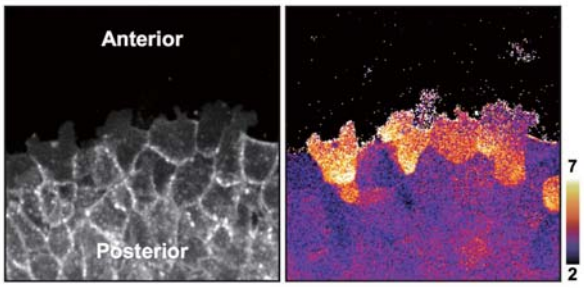

Figure 1. A snapshot of intracellular Ca^{2+} transients in a migrating LEM explant of *Xenopus laevis*. Ca^{2+} dynamics were visualized with a FRET probe YC-Nano2. Inhibition of these Ca^{2+} transients with Ca inhibitors perturbs the tissue migration.

II. Mechanotransduction in *Xenopus* embryonic cells

During early embryogenesis, a variety of dynamic morphogenetic movements occur, which include convergent extension of the axial mesoderm, epiboly of the ectoderm, and neural tube formation. These movements must generate physical forces at the levels of cells and tissues. However, it is still not fully understood how these forces influence morphogenetic processes. This project is to elucidate the mechanisms of sensing and responding to mechanical stimuli in *Xenopus* embryos.

Cells are known to sense mechanical stresses in several ways, for example, with TRP channels, F-actin, cadherins, and focal adhesions. Physical stimuli sensed by these molecules are converted to intracellular chemical signals, which in turn induce cellular response. Protein phosphorylation may be one of the earliest responses to mechanical stresses. Therefore, we are attempting to profile protein phosphorylation upon mechanical stimuli and identify target proteins in *Xenopus* embryos. In order to comprehensively analyze levels of protein phosphorylation, we took a phosphoproteomic approach using mass spectrometry. So far, we found that a mechanical stress applied to *Xenopus* embryos changes phosphorylation levels of many proteins related to cytoskeleton and cell adhesion. Consistently, we observed that mechanical stimulation induced cytoskeletal remodeling. Furthermore, some protein kinases, including ERK2, were activated in response to a mechanical force. As shown in Figure 2, ERK2 was phosphorylated by the mechanical force and its phosphorylation was rapidly attenuated after release from the force. We concluded that *Xenopus* embryos have a system to sense and respond to mechanical stimulation, which activates protein kinase signaling pathways and regulates cytoskeletal organization.

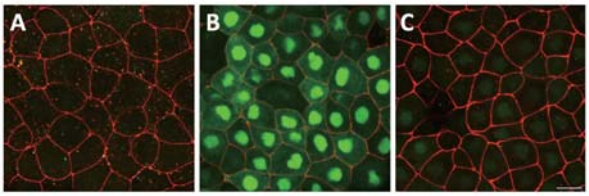

Figure 2. ERK2 is activated by a mechanical force in *Xenopus* embryonic cells.
A mechanical force was applied to *Xenopus* gastrula embryos by centrifugation. Embryos were stained with anti phoshorylated ERK2 antibody (green) and an actin-binding compound, phalloidin (red). Superficial cells around the animal pole were observed. (A) control (not centrifuged). (B) centifuged at 450 x g for 15 min. (C) centrifuged as in B and then incubated for 15 min. Bar = 25 μm

III. Statistical analysis of neural tube formation based on pulsed apical constriction

During early development of the central nervous system, neuroepithelial cells constrict their apices by actomyosin contractility. This cell shape change is called apical constriction, which makes the neural plate into a tubular structure, called the neural tube. We previously showed that two types of intracellular Ca^{2+}-concentration changes, a single-cell and a multicellular wave-like fluctuation occurred in the *Xenopus* neural plate and modulate the patterns of apical constriction during neural tube formation. In this study, to further investigate the role of the Ca^{2+}-induced pulsed apical constriction, we performed statistical analysis in which we fitted a linear mixed model with single-cell and multicellular Ca^{2+} transients as explanatory variables and the amount of the closing movement of the neural tube as a response variable to data of wild-type embryos, and obtained estimated contributions of single-cell Ca^{2+} transients and multicellular Ca^{2+} transients. We found that the coefficient of single-cell Ca^{2+} transients was significantly larger than that of multicellular Ca^{2+} transients. These results suggest that the Ca^{2+} fluctuations at the single-cell level more effectively accelerate apical constriction and neural tube formation than do multicellular Ca^{2+} fluctuations *in vivo*.

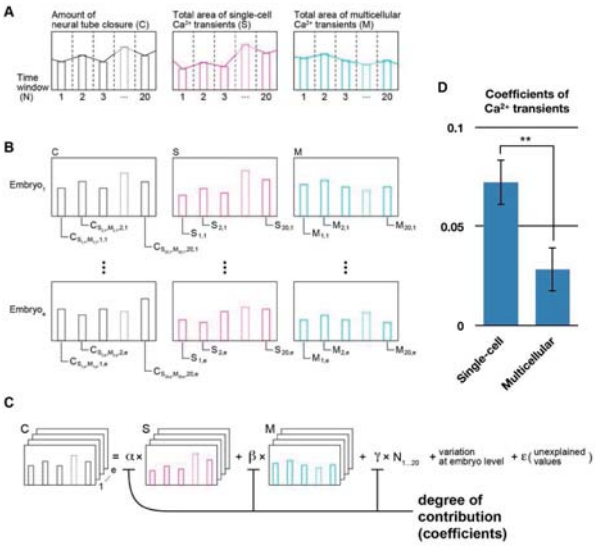

Figure 3. Schematic workflow and coefficients of the statistical model.
(A) Temporal profiles of closing movement, the total area of single-cell Ca^{2+} transients, and multicellular Ca^{2+} transients during neural tube formation. (B) Generation of data set for statistical analysis by dividing temporal profiles into time windows. (C) Fitting mixed linear model to the data set to estimate contributions. (D) Statistics for fixed effects.

IV. A novel membrane structure orchestrates centrosome positioning and the orientation of cell division axis

The positioning of the centrosome is critical for the polarity of cilia and the orientation of the mitotic spindle, which are important events during morphogenesis. Previously, in ascidian, *Ciona intestinalis* embryos, we found a unique centrosome-targeting of the membrane structure (membrane invagination) in epidermal cells, which divide stereotypically along the anterior-posterior (A–P) axis. We also reported that membrane invaginations showed the A–P polarity and a tensile force toward the posterior end of cell. By injection of antisense morpholino oligo (MO), we revealed that Dishevelled (Dsh), a core component of Planar Cell Polarity (PCP) pathway was involved in the A–P polarity of membrane invaginations. In the Dsh-depleted embryos, the direction of the invagination as well as mitotic spindle orientation was randomized. In addition, epidermal cilia normally localized in the posterior side of the cell was anteriorized. These results support our hypothesis that the membrane invagination controls the direction of cell division as well as ciliary positioning via the tethering centrosome at the posterior side of the epidermal cell and highlight physiological importance of this unique structure.

V. Notochord and evolution of chordates

The notochord is the most prominent organ in chordates in which *Brachyury* (*Bra*) plays pivotal roles in its formation. Since *Bra* is shared by non-chordate animals and expressed in the blastopore it is an intriguing EvoDevo question how *Bra* acquired enhancers that promote its gene expression in the notochord as well as in somatic muscles during the evolution of chordates. In cephalochordate lancelets *Bra* is duplicated. We examined enhancer activity of *Branchiostoma floridae Bra* (*BfBra1* and *BfBra2*) using *Ciona* egg electroporation system. Vista analysis suggested the presence of conserved non-coding sequences not only in 5'-upstream but also 3'-downstream and introgenic regions. The *lacZ* reporter assay showed that (1) the 5'-upstream sequences of both genes promoted reporter expression in muscle cells, (2) the 3'-downstream sequences also have enhancer activity and promoted *lacZ* expression in notochord cells, (3) the introgenic regions of *BfBra2* and *BfBra1* exhibited the activity in muscle and notochord cells, respectively, and (4) the enhancer activity appeared as early as the gastrula stage. These results indicate that the 5', 3' and introgenic regions work together to enhance the *Bra* expression in muscle and notochord cells. The acquisition of enhancer activity of *BfBra* is highly likely to be involved in the formation of muscles and notochord in chordate embryos, which in turn led to the evolution of chordates.

VI. Gene order and direction of transcription contribute to the small genome size in *Ciona intestinalis*

DNA methylation at cytosine residues is an important epigenetic modification found in eukaryotes ranging from plants to humans. Invertebrates offer an interesting model for studying evolutionary changes in the targets and the function of DNA methylation. A marine invertebrate chordate *Ciona intestinalis* has a genome-wide mosaic methylation pattern comprising methylated and unmethylated genes. It has been observed that DNA methylation is targeted to the transcribed region of ubiquitously expressed genes, and a constant targeting of "gene body methylation" irrespective of cell types. To reveal the function of gene body methylation in gene transcription, we analyzed newly synthesized RNA from *C. intestinalis* embryos. By using 4sU labeling and sequencing methods, revealing global RNA processing kinetics at nucleotide resolution, we obtained snapshots of active transcription. Significant differences were seen in co-transcriptional splicing efficiency, in connection with methylation status of exons and introns. The splicing efficiency and DNA methylation status were also correlated to nucleosomal positions, suggesting that epigenetic states in the bodies of transcribed genes control the pre-mRNA processing through nucleosomal positioning.

VII. Effects of light on larval swimming behavior in scleractinian corals

Many reef-building corals form a symbiotic relationship with dinoflagellate algae of the genus Symbiodinium. Corals mostly depend on photosynthetic products from these symbionts for their energy source, and thus light conditions in habitats can influence post-settlement survival. Several previous studies reported that light environments play an essential role in larval habitat selection. However, due to lack of basic photobiological studies in corals, how they sense light and how light affects biological tasks remain largely unexplored. The purpose of this study is to reveal the mechanism of phtoreception and clearly understand the effects of light on reproduction and behavior in corals.

For this purpose, we focused on the effects of light on larval swimming behavior of Acropora corals. We analyzed the larval swimming activity with different light stimuli, and found that larvae paused their swimming behavior immidiately after a change in intensity and spectral composition of light. In addition to the behavioral assay, we surveyed the genome of coral, *Acropora digitifera* and *A. tenuis*, for detecting photoreceptor opsin genes using a molecular phylogenetic analysis. Five opsin gene orthologs were found on the genome and two of the five genes were transcribed in the coral larvae. These results suggested that coral larvae sense light with an opsin-based phototranduction cascade and change their behavior responding to the rapid change in light environments.

Figure 4. A view of stony corals around Orpheus Island, Queensland Australia, November 2017

Publication List:

〔Original papers〕

- Murakami, F., Ando, Y., Miyagi, A., Sugita, S., Ueno, N., and Matsumoto, T. (2017). Measurement of surface topography and stiffness distribution on cross-section of Xenopus laevis tailbud for estimation of mechanical environment in embryo. Dev. Growth Differ. *59*, 343-443.
- Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Cambell, R.E., and Ueno, N. (2017). Distinct intracellular Ca2+ dynamics regulate apical constriction and differentially contribute to neural tube closure. Development *144*, 1307-1316.
- Tanaka, T., Ochi, H., Takahashi, S., Ueno, N., and Taira, M. (2017). Genes coding for cyclin-dependent kinase inhibitors are fragile in Xenopus. Dev. Biol. *426*, 291-300.
- Tokue, M., Ikami, K., Mizuno, S., Takagi, C., Miyagi, A., Takada, R., Noda, C., Kitadate, Y., Hara, K., Mizuguchi, H., Sato, T., Taketo, M.M., Sugiyama, F., Ogawa, T., Kobayashi, S., Ueno, N., Takahashi, S., Takeda, S., and Yoshida, S. SHISA6 confers resistance to differentiation-promoting Wnt/β-catenin signaling in mouse spermatogenic stem cells. Stem Cell Rep. *8*, 561-575.

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The morphology of the body and tissues is established in spatio-temporarily regulated manners. A number of genes involved in morphogenesis have been identified, but it is still uncertain how the spatial and temporal information are established and converted to morphology during embryogenesis. Therefore, we are challenging to understand the mechanism by which the spatial information is established and that by which the temporal, or periodical, information is converted into morphology by several different approaches.

In the development of many tissues, secreted signal molecules are important for the formation of spatial information. These molecules are secreted from producing cells and transported to surrounding cells, resulting in the formation of concentration gradients. Given that their concentration decreases according to the distance from the source, the gradient of the signals defines relative positions of receiving cells in developing tissues. Many genetic studies revealed that a limited number of secreted signal proteins, including Wnt, BMP, and Hedgehog, function in the morphogenesis of tissues and embryos. In spite of the accumulation of genetic evidence, however, the molecular mechanism that regulates their spread in particular developing tissues remains to be elucidated. To t his end, we started to visualize signal proteins and monitor their movement in tissues. In addition, we are examining structural and biochemical characteristics of these molecules, which appear to affect how they spread.

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

I. Spatial regulation of secreted Wnt proteins in vertebrate development

The Wnt family of secreted signal proteins plays a key role

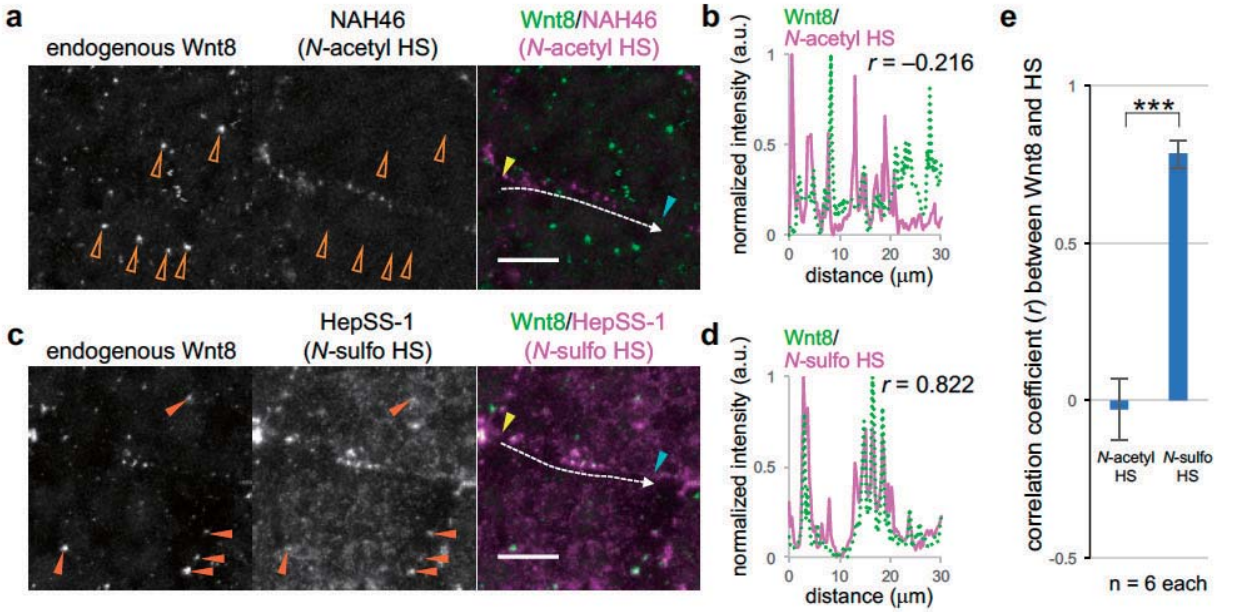

Figure 1. Colocalization of Wnt8 and N-sulfo-rich HS clusters. **a**-**d**, The colocalization of endogenous Wnt8 and a subpopulation of HS. Gastrula embryos (st. 10.5) were coimmunostained for Wnt8 and NAH46 epitopes (*N*acetyl HS) or HepSS1 epitopes (*N*sulfo HS) at the ventral marginal zone (**a**, **c**). Signal intensities along white arrows were plotted (**b**, **d**), starting and ending points as indicated by yellow and cyan arrowheads, respectively, in **a** and **c**. Correlation coefficients (*r*) for the plots were as indicated. Of note, Wnt8 puncta inside cells were overlapped with *N*sulforich HS clusters (orange arrowheads in **b**) but not with *N*acetyl-rich HS clusters (open arrowheads in **a**). Scale bars, 10 μm. **e**, A high correlation between distributions of Wnt8 and *N*-sulfo HS. Correlation coefficients of distributions between Wnt8 and *N*-sulfo HS were significantly higher than that between Wnt8 and *N*-acetyl HS (mean +/– s.d. ***$p < 0.001$, t-test).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

in numerous aspects of embryogenesis. In a classical view, it has been proposed that secreted signal proteins, like Wnt proteins, spread and function over a distance of several cells in many aspects of morphogenesis. In contrast, accumulating evidence also implies that Wnt proteins appear to transmit their signals locally in some particular cases. Therefore, their secretion and transport might be differently controlled depending on situation. Thus, for understanding the spatial regulation of tissue morphogenesis, the molecular mechanism underlying the spreading of Wnt proteins should be revealed.

For better understanding the spreading of Wnt proteins, we started to examine the characteristics of Wnt proteins both *in vitro* and *in vivo*. Since Wnt proteins are easily assembled during conventional biochemical analysis, we are trying to utilize some non-invasive methods for this characterization. Our preliminary study indicated that Wnt proteins are secreted by forming specific complexes. In addition, analysis of the mobility of Wnt complexes in the extracellular space suggested that Wnt proteins are not simply diffused during embryogenesis. We precisely examined regulatory mechanisms and biological significance of Wnt protein distribution in mouse embryos. These analyses revealed a novel view of spatial regulation of Wnt signaling.

Extracellular molecules are known to regulate special distribution of Wnt proteins. Once Wnt proteins are secreted from the producing cell, their spread is regulated through interaction with these molecules. The heparan sulfate proteoglycan (HSPG), which is composed of a core protein with several chains of HS glycosaminoglycans, is a major component involved in this interaction. In collaboration with Prof. Taira at the University of Tokyo, we found that HSPGs with different sugar chain modifications form distinct extracellular structures, which are called heparan sulfate nanostructures (HSNSs), in the early *Xenopus* embryo.

We first examined the distribution of the endogenous Wnt8 protein in *Xenopus* embryos. Wnt8 shows a gradient in the embryo, but at the same time, it is accumulated on the cell surface in a punctate manner. Accumulated Wnt8 is colocalized with a specific state HS, the sulfonated state of HS (*N*-sulfo HS; Figure 1). Furthermore, we found that the default or non/less-sulfonated state (*N*-acetyl HS; Figure 1a, b) and the sulfonated state of HS (*N*-sulfo HS; Figure 1c, d) are separately clustered. *N*-sulfo HS clusters with accumulated Wnt8 recruit the receptor Frizzled (Fzd) and the transducer Dishevelled (Dvl), possibly forming a signaling complex called the signalosome or multivesicular body, suggesting a direct connection between the distribution of Wnt and signalling. In contrast *N*-acetyl HS cluster can accumulate the secreted Wnt inhibitor Frzb. With overexpressed Frzb, Wnt8 is trapped by *N*-acetyl HS cluster, which is suggested to contribute to antagonism of Wnt signaling. We further demonstrate that Gpc4, a Dlp-ortholog forms both *N*-sulfo and *N*-acetyl HS while Gpc5, a Dally-ortholog forms *N*-sulfo HS. Thus, our finding of discrete clusters of HS provide a regulatory basis for the distribution and signaling of Wnt, discussed above. We further demonstrate that internalization of *N*-sulfo HS is more frequent than that of *N*-acetyl HS, which could explain the differences between the short- and long-range distributions of Wnt and Frzb, respectively and also range-expansion of Wnt8 by Frzb (Figure 2) .

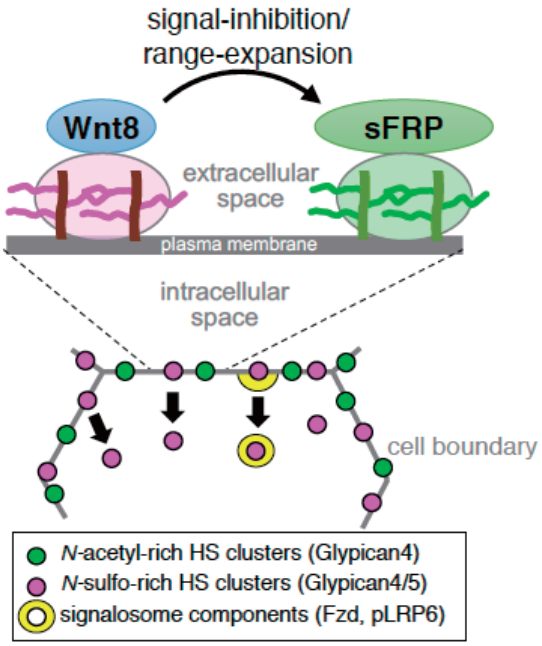

Figure 2. Model of the *N*sulforich and *N*acetylrich HS cluster system for the regulation of the extracellular distribution and signal reception of the Wnt morphogen. The two types of HS clusters, *N*-sulfo-rich and *N*-acetyl-rich HS clusters differ in several aspects including core proteins, tendency of internalization and capability of signaling. Together with the specificity of Wnt8 to the *N*-sulfo-rich HS clusters and that of Frzb to the *N*-acetyl-rich HS clusters, the two types of HS clusters could serve as a system to regulate the extracellular distribution and signal reception of the Wnt morphogen.

II. Molecular mechanism of somite development

Somites are segmental epithelial blocks located symmetrically on either side of the neural tube. Somites are periodically generated in an anterior to posterior manner from their precursor, the presomitic mesoderm (PSM), which is located posterior to newly formed somites. This periodic generation is achieved by a complex and dynamic mechanism in the PSM. First, the molecular clock, the so-called segmentation clock, creates oscillatory expression of particular genes, *hairy* and some *notch*-related genes, in the posterior PSM. Because the phase of oscillation is gradually shifted along the posterior-to-anterior axis, a wave of the oscillation appears to move in a posterior-to-anterior fashion. This oscillatory gene expression subsequently resulted in periodical generation of morphologically segmented somites.

The spatial pattern of somites is defined by positioning of intersomitic boundaries and rostro-caudal patterning within a somite. This spatial pattern is established in the anterior PSM by converting the oscillatory gene expression into spatially restricted patterns of gene expression. Several intra-cellular molecules, as well as FGF and Wnt signaling, are involved in this conversion. It has been generally considered that Mesp of bHLH transcriptional regulator plays a critical role in this conversion. In the mouse, the prospective segmentation boundary is periodically established in the anterior

PSM at the rostral border of the *Mesp2* expression domain. In contrast, recent studies by us and other groups strongly suggested that Mesp2 does not directly define the position of the segmentation boundary, rather other genes called *Ripply1* and *Ripply2* play more essential roles in this conversion in the mouse and zebrafish. *Ripply* genes encode ~100 amino acid proteins, which commonly possess 2 distinct amino acid sequences: a highly conserved WRPW stretch and a conserved ~50-amino acid stretch, called the Ripply homology (RH) domain, that interacts with the T-box proteins, including Tbx6, which is involved in the positioning of the segmentation boundary. Currently, we are examining the mechanism of this conversion by focusing on the regulation of Ripply's function in zebrafish.

III. Molecular mechanism of pharyngeal pouches

In addition to somites, metameric structures are observed in the pharyngeal region of vertebrates. Typical examples of such structures are skeletal elements of jaws, gills and cranial nerve projections. This metamerism is brought about by the segmental development of the pharyngeal pouches, which are generated by outpocketing of the pharyngeal endoderm. However, the molecular mechanisms underlying the segmentation of the pharyngeal pouches and the morphogenesis of the pharyngeal pouches still remained to be elucidated.

To understand the mechanism of the development of pharyngeal pouches, we sought to examine mouse embryos defective for *Ripply3*, a member of the Ripply family. In mouse embryos, *Ripply3* is specifically expressed in the pharyngeal endoderm and ectoderm. We previously showed that *Ripply3*-deficient embryos fail to develop the pharyngeal arches posterior to the 2nd pharyngeal pouch. However, the molecular and cellular mechanisms by which Ripply3 regulates the morphogenesis of pharyngeal pouches have still been unclear.

To better understand the cellular process mediated by *Ripply3*, we sought to identify the primary defect observable in *Ripply3*-deficient mouse embryos. First, by following *Ripply3* expression during the formation of the 3rd pharyngeal pouch, we found that *Ripply3* expression was correlated with the position where epithelial bending took place. In addition, precise histological analysis at short-term intervals revealed that *Ripply3* was required for the maintenance of a continuous monolayer of pharyngeal epithelial sheets during their bending. Further analyses using *Ripply3*-deficient embryos and Ripply3-expressing culture cells supported an idea that Ripply3 promotes maturation of adhesive contacts transmitting mechanical force between the extracellular matrix and the interacting cells. Based on these results, we propose a model in which Ripply3 plays a role for the resistance against the mechanical stress in epithelial bending probably by enhancing the attachment of the pharyngeal endoderm cells to the extracellular matrix.

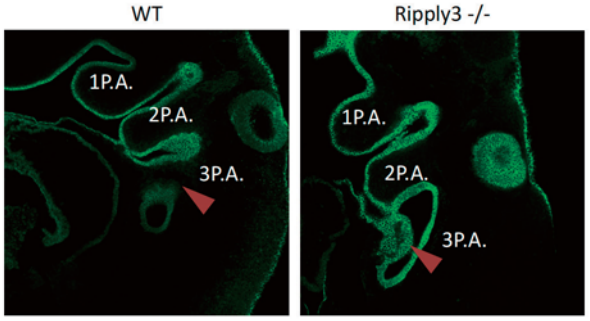

Figure 3. Ripply3 is required for the proper morphogenesis of the posterior pharyngeal pouches in the mouse embryo. The formation of the 3rd pharyngeal arch (red arrowhead) was impaired in Ripply3 deficient embryos.

Publication List:

〔Original papers〕

- Mii, Y., Yamamoto, T., Takada, R., Mizumoto, S., Matsuyama, M., Yamada, S., *Takada, S., and *Taira, M. (*Co-corresponding authors) (2017). Roles of two types of heparan sulphate clusters in Wnt8 distribution and signalling in Xenopus. Nat. Commun. *8,* 1973.
- Tokue, M., Ikami, K., Mizuno, S., Takagi, C., Miyagi, A., Takada, R., Noda, C., Kitadate, Y., Hara, K., Mizuguchi, H., Sato, T., Taketo, M.M., Sugiyama, F., Ogawa, T., Kobayashi, S., Ueno, N., Takahashi, S., Takada, S., and Yoshida, S. (2017). SHISA6 confers resistance to differentiation-promoting Wnt/β-catenin signaling in mouse spermatogenic stem cells. Stem Cell Rep. *8,* 561-575.

〔Review article〕

- Takada, S., Fujimori, S., Shinozuka, T., Takada, R., and Mii, Y. (2017), Differences in the secretion and transport of Wnt proteins. J. Biochem. *161*, 1-7.

Division of Embyology

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The aim of our research is to understand the events underlying early mammalian development. One of the characteristics of mammalian embryonic development is that embryogenesis proceeds in the oviducts and the uterus of the mother, and interaction between an embryo and maternal tissue is essential. Another characteristic is the highly regulative potential of embryos. The pattern of cell division and allocation of cells within an embryo during the early stages vary between embryos. The timing of the earliest specification events that control the future body axes is still under investigation. We want to provide basic and fundamental information about the specification of embryonic axes, differentiation of cell lineages, behaviors of cells, and the regulation of body shape and tissue morphology in early mammalian development. We are also interested in the relationship between embryos and their environment, the oviducts and the uterus.

I. Live observation of early mouse development

To understand the mechanisms underlying embryonic development and morphogenesis, it has become common to observe the behavior of cells and gene expression in living embryos in a variety of animal species. Progress in genetic engineering and embryonic manipulation concomitantly with the progress in optics and microscope technologies, including development of highly sensitive photo-detectors, has allowed us to observe developing embryos live, even in mammals. We have established a series of transgenic mouse lines for live imaging, which is part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CLST. These mouse lines have been used to visualize nuclei, cell shapes, the cytoskeleton and other organelles to observe cell behaviors in living mouse embryos in many laboratories over the world. We also established mouse lines to monitor the cell cycle.

We have also been establishing several reporter mouse lines in the lab to study gene expression patterns during the peri-implantation stage of mouse development. In these mice, cDNA encoding fluorescent proteins are connected to the mouse genomic DNA sequences of the enhancer/promoter region of important genes encoding factors regulating cell differentiation in these stages. Combining these two types of reporter transgenic mouse lines, we have been analyzing behaviors of cells comparing gene expression properties at the single cell level. We found that cells expressing Cdx2 exhibit plasticity of specification to TE and ICM lineages through positional changes.

II. Histological observation of mouse embryos developing in the uterus

Mammalian embryos develop in the uterus after implantation. Direct interaction between embryos and the cells of the uterus is important. In studies on developmental biology of mammalian embryos, embryos are usually removed from the uterus, and isolated embryos are analyzed. We have been analyzing early embryonic development of mouse comparing the changes in the uterine epithelium. To observe the morphogenesis of embryos within the uterus, serial sections of implanted uteruses were made, and images of the embryos within the uteruses were captured to make high resolution three-dimensional re-constructions. We are preparing samples obtained from various developmental stages. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic development have been examined using these images. We are identifying molecules involved in the interaction between embryo proper and uterine cells, which may play major roles in embryonic development.

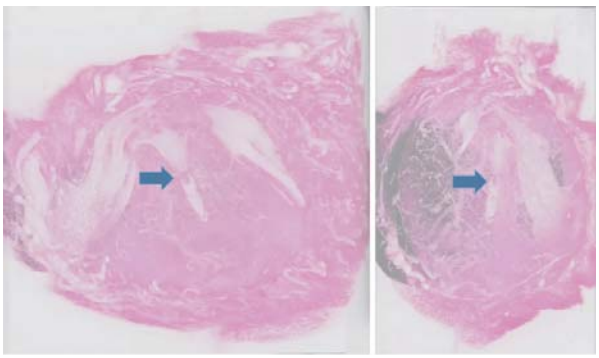

Figure 1. Three-dimensional image reconstruction of the pregnant uterus. Uterus of 5 days after fertilization were sectioned and images were digitized by image scanner, and reconstructed three-dimensionally to observe the relationship between embryo and the maternal tissue. Right panel represents the pseudo 3D image observed from the lateral side of the left image. Arrows indicate the embryo in the decidual tissue.

III. Polarity formation in the mouse oviduct epithelium

The oviduct (Fallopian tube) is the organ connecting the periovarian space and the uterine horn. The ova released from the ovary are transported through the oviduct, where the ova are fertilized by spermatozoon entering from the uterus. The epithelium of the mouse oviduct consists of multi-ciliated cells and secretory cells. Ovulated oocytes are transported in oviducts by unidirectional motility of multicilia and the resultant secretory fluid flow from ovary to uterus. This cellular orientation in the oviduct is one example of planar cell polarity (PCP). PCP is seen in a variety of tissues, where cells sense global axes of the tissue to which

they belong and orient themselves to fulfill specialized functions.

Many evolutionally conserved genes are required for the formation of PCP, and several of those encode proteins that are localized in polarized manners within cells. We found that Celsr1, a PCP core member, was strongly concentrated only at the specific domains of cell boundaries which are perpendicular to the ovary-uterus axis and that this polarized localization appeared to precede the directional movement of cilia.

In *Celsr1*-deficient mutant oviducts, the beating direction was not coordinated along the ovary-uterus axis throughout the epithelia; some cells were oriented orthogonally to the axis, while some others exhibited reverse polarity. In addition to the ciliary polarity phenotype, cellular shape polarity was also impaired in the *Celsr1*-deficient mice. We found a new feature of cellular polarity in the wild type oviduct, e.g. that the apical surface of epithelial cells was elongated along the ovary-uterus axis. In *Celsr1*-deficient mice, epithelial cells showed less elongation and randomized orientation.

Furthermore, we also found that the three-dimensional architecture of oviductal epithelia was dramatically disrupted. In adult wild-type mice, the epithelial cell sheet is bent multiple times, which forms around twenty longitudinal folds parallel to the organ axis. However, in the mutant oviducts, epithelial folds no longer run parallel to the organ axis. The direction of the folds was randomized, and ectopic branches of the folds were observed. This suggests that Celsr1 is important for the formation of multi-scale polarities in the mouse oviduct ranging from the cellular to the tissue scale.

To investigate the mechanisms of the epithelial fold pattern formation, we utilized mathematical modeling and simulations. By considering mechanical properties of the epithelial sheets we reproduced the longitudinally aligned folds and the branched folds which are observed in wild-type and the *Celsr1* mutant mice, respectively (Figure 2). Experimental measurements of mechanical tensions in the epithelial sheet were consistent with the tensions predicted from the simulations. Our experimental and mathematical analyses also successfully linked the epithelial tensions to cellular shapes. We are also focusing on some other PCP regulators, and are

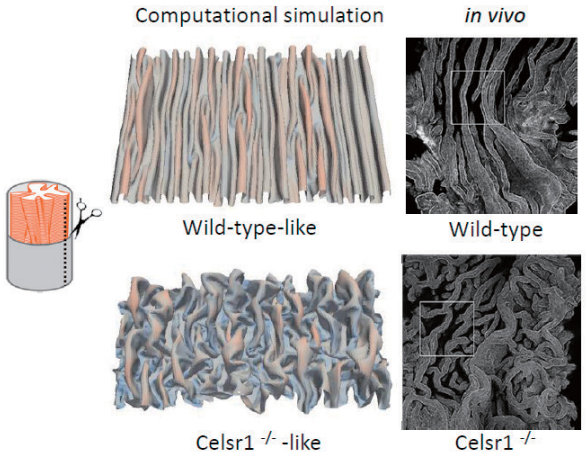

Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

trying to understand how oviduct epithelial cells establish and maintain their polarity.

IV. Analysis of mechanical properties of cells and tissues during embryonic development

Mechanics is one of the essential components of biological processes, including cell shape transformation and tissue morphogenesis etc. To understand how mechanical forces contribute to various patterns of morphogenesis, measuring cellular and tissue mechanical states is necessary. We developed statistic techniques to infer mechanical states using fluorescent microscopic images during morphogenesis (Figure 3). By employing this method, we inferred mechanical forces in multi-cellular systems including cultured epithelial cells, and early embryogenesis in *C. elegans* and mice. Further computational simulations based on the inferred mechanical information reproduced morphological features of the multi-

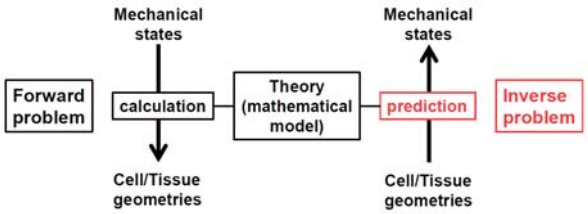

Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

cellular systems. Thus, the mechanical information will be useful to investigate physical mechanisms of early embryonic development and morphogenesis during organogenesis in late stages of development.

V. Mammalian tissue morphogenesis requiring mechanosensor channel Piezos

Several examples have shown that mechanical stimuli can work as key components for tissue or organ development. However, our knowledge about involvement of mechanotransduction in biological phenomena or their precise mechanisms is still limited. It is partially because key mechanosensors are not yet identified in many cell types. Piezos are recently identified mechanically activated cation channels functioning in mammalian cells (Figure 4). They are activated when mechanical forces are applied on the cell membrane. Series of data show that Piezo2 serves as the main mechanosensor in sensory neurons for light touch sensation, proprioception and breathing. We found that Piezos are also required for proper vascular morphogenesis. To further elucidate how Piezo-mediated mechanotransduction is involved in vascular development, we have been developing systems to manipulate mechanical stimuli and monitor

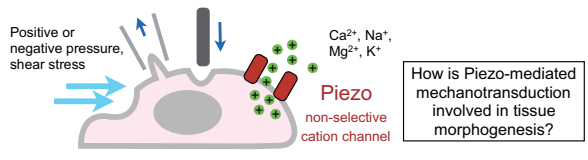

Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

Piezo activity in vitro and in vivo. Analyses utilizing these systems and mouse lines deficient in Piezos will clarify the relationship among mechanical forces, Piezo activation, cellular responses, and tissue morphogenesis, this may provide a basis to understand the roles of mechanotransduction in tissue morphogenesis.

VI. Mechanics of cell population patterning during development

During development, cells actively and/or passively move, resulting in various patterns of cell distribution. We investigated the effect of both active and passive cell movements. First, we mathematically modeled the process of neural tube closure in *Xenopus laevis*, and found that active cell contractility and its frequency are critical parameters for the tissue shrinkage. Second, we theoretically investigated the effect of passive cell movements provoked by frictional forces from surrounding tissues. The passive movements generated various patterns, such as an elongated cell cluster, multiple cell clusters, etc. The former situation is actually observed during elongation of the notochord in mice. This theoretical framework would be widely applicable to developmental processes.

Publication List:

〔Original papers〕

- Minegishi, K., Hashimoto, M., Ajima, R., Takaoka, K., Shinohara, K., Ikawa, Y., Nishimura, H., McMahon, A.P., Willert, K., Okada, Y., Sasaki, H., Shi, D., Fujimori, T., Ohtsuka, T., Igarashi, Y., Yamaguchi, T.P., Shimono, A., Shiratori, H., and Hamada, H. (2017). A Wnt5 activity asymmetry and intercellular signaling via PCP proteins polarize node cells for left-right symmetry breaking. Dev. Cell *40*, 439-452.
- Nonomura, K., Woo, S.H., Chang, R.B., Gillich, A., Qiu, Z., Francisco, A.G., Ranade, S.S., Liberles, S.D., and Patapoutian, A. (2017). Piezo2 senses airway stretch and mediates lung inflation-induced apnoea. Nature *541*, 176-181.
- Shioi, G., Hoshino, H., Abe, T., Kiyonari, H., Nakao, K., Meng, W., Furuta, Y., Fujimori, T., and Aizawa, S. (2017). Apical constriction in distal visceral endoderm cells initiates global, collective cell rearrangement in embryonic visceral endoderm to form anterior visceral endoderm. Dev. Biol. *429*, 20-30.
- Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Campbell, RE., and Ueno, N. (2017). Distinct intracellular Ca^{2+} dynamics regulate apical constriction and differentially contribute to neural tube closure. Development *144*, 1307-1316.

DIVISION OF GERM CELL BIOLOGY

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Overview of our research

Production of numerous sperm for a long period in testis is fundamental for continuity of life across generations. This process of spermatogenesis is supported by the robust function of "stem cells", which both maintain the undifferentiated cell pool, while generating differentiation-destined cells in a well balanced manner. The Division of Germ Cell Biology aims to fully understand the mouse sperm stem cell system under the context of *in vivo* testicular tissue. Our particular interests have been laid on the "undifferentiated spermatogonia", which are responsible for the stem cell functions. Our study has revealed several key properties of this interesting population.

First, we found that this stem cell system includes a functional hierarchy. It is comprised of an "actual" stem cell compartment that is prone to self-renew, and a differentiation-primed, "potential" stem cell compartment. Regarding the "actual" stem cells, we have been investigating their cellular identity, their *in vivo* behavior at a single-cell resolution, and the underlying mathematical principles. This lead to the discovery of "neutral competition" between the stem cells. We are currently investigating the molecular mechanism underlying their control of self-renewal and differentiation, and their connection to tissue environment. "Potential stem cells" are also of our enthusiastic interest: In undisturbed, steady-state spermatogenesis, they largely contribute as transient amplifying cells and differentiate rather than self-renew. However, when tissues are insulted or testicular cells are transplanted to a host testes, their probability of self-renewal jumps up and they effectively replenish the lost "actual" stem cells. Such a flexible, and probabilistic, feature of stem cell dynamics has been found paradigmatic for many other stem cell-supported tissues.

Key references from this division related to these studies that are currently public include Nakagawa et al., Dev. Cell 2007; Yoshida et al., Science 2007; Nakagawa et al., Science 2010; Klein et al., Cell Stem Cell 2010; and Hara et al., Cell Stem Cell 2014, Ikami et al., Development 2015, and Tokue et al., Stem Cell Reports 2017.

I. The identity of spermatogenic stem cells and their dynamics

Morphologically, the population of A_{undiff} includes singly isolated cells (A_s), or syncytia consisting mainly of 2 (A_{pr}), 4 ($A_{al\text{-}4}$), 8 ($A_{al\text{-}8}$), or 16 ($A_{al\text{-}16}$) cells. The formation of syncytia is due to incomplete cell division, a germline-specific mitotic division process with which cytokinesis does not complete and the cytoplasmic connection between daughter cells persists via intercellular bridges. The prevailing stem cell theory proposed in 1971 states that stem cell activity is restricted to the population of A_s cells, while interconnected A_{pr} and A_{al} syncytia are irreversibly committed to differentiation and no longer contribute to the stem cell pool (Huckins, 1971; Oakberg, 1971), known as the "A_s model".

Figure 1 represents our latest proposed model for the functional structure of the spermatogenic stem cell system, which indeed proposes an alternative for the "A_s model". This is the simplest interpretation of the results of our functional analyses of GFRα1$^+$ spermatogonia, which act as the "actual" stem cells. These include intravital live-imaging experiments, clonal fate analysis of pulse-labeled cells, and biophysical modeling analysis of the results.

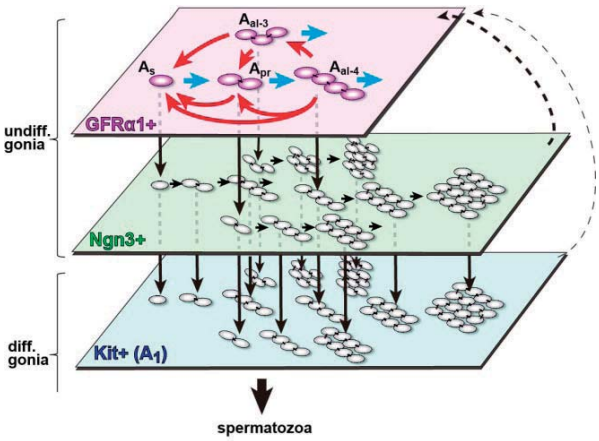

Figure 1. A proposed stem cell dynamics. On the top of the differentiation hierarchy, GFRα1$^+$ spermatogonia comprise a single stem cell pool, in which cells continually and reversibly interconvert between states of A_s, A_{pr} and A_{al} spermatogonia through incomplete cell division (blue arrows) and syncytial fragmentation (red arrows), while giving rise to NGN3$^+$ cells. After leaving the GFRα1$^+$ compartment, differentiation-destined cells follow a series of transitions (GFRα1$^+$→NGN3$^+$→KIT$^+$; downward black arrows) that accompany the extension of syncytial length (rightward black arrows). Ngn3$^+$ and, to a lesser extent, KIT+ cells retain the capacity to revert back into the GFRα1+ compartment in a context-dependent fashion (broken arrows). (Reprinted from Hara et al., Cell Stem Cell 2014.)

As crystalized in this model, our results suggest that the GFRα1$^+$ sub-population of A_{undff} spermatogonia, which include both A_s cells and syncytia (A_{pr} and A_{al}) comprises a single stem cell pool, in which cells continually interconvert between these morphologically heterogeneous states through stochastic incomplete division and fragmentation of syncytia. The incomplete division and syncytial fragmentation causes the expansion of this population, while the excess cells over

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

a particular "quota" would overflow to become the NGN3$^+$ state of A_{undff}, which then further differentiate into KIT$^+$ "differentiating" spermatogonia that are largely devoid of self-renewing potential.

Currently, we are investigating the mechanism that determines the quota or tissue capacity of GFRα1$^+$ cells, as well as the detailed nature of the internal heterogeneity of the population of GFRα1$^+$ cells. Further, it is also becoming apparent that the GFRα1$^+$ population is not totally homogeneous but associated with some heterogeneity in their gene expression and fate behavior, as described by our group (see below) and others. Therefore, our next challenges include understanding the overall scheme of the stem/progenitor cell dynamics in which short-term heterogeneity and long-term equipotency (randomness) are both observed in a compatible manner.

2. Mechanisms underlying the balanced differentiation and self-renewal of stem cells in an "open" stem cell niche

In general, stem cells support tissue homeostasis through continual production of differentiating progeny from a pool of undifferentiated cells. This is traditionally thought to be dependent on a couple of paradigmatic mechanisms: 1) "asymmetric cell division", which always gives rise to one self-renewing cell and one differentiating cell; 2) control by "an anatomically defined niche", inside of which stem cells remain undifferentiated, but outside of which they differentiate (Fuller and Spradling, 2007; Morrison and Spradling, 2008). However, the mouse spermatogenic stem cell system appears to not show a defined facultative niche environment and does not rely on asymmetric division; their fates are found to be stochastic (Hara et al., 2014; Klein et al., 2010; Klein and Simons, 2011).

In 2017, we challenged a fundamental question: How sperm stem cells follow different fates (to differentiate or to self-renew) in response to homogeneously distributed extracellular signals, in a facultative (or open) niche environment? The result is published in Tokue et al., Stem Cell Reports (2017).

2-1. Wnt/β-catenin signaling promotes differentiation of GFRα1$^+$ to NGN3$^+$ cells

To unveil the signal that promotes the GFRα1$^+$ to NGN3$^+$ transition. We first compared the gene expression profiles between GFRα1$^+$ and NGN3$^+$ cells by cDNA microarray. From the microarray data, we focused on Wnt signaling. In cultured spermatogonia (viz. GS cell), Wnt/β-catenin signaling induced *Ngn3* expression (Figure 2A). Moreover, mice carrying a gain-of-function mutation of *β-catenin* showed spermatogenesis defects, in which the number of GFRα1$^+$ cells was reduced (Figure 2B), whereas no significant reduction in retinoic acid receptor gamma (RARγ)$^+$ cells (largely corresponding to NGN3$^+$ cells) was observed. Consequently, the RARγ$^+$ cell-to-GFRα1$^+$ cell ratio increased, consistent with the idea that GFRα1$^+$ to NGN3$^+$ differentiation increased.

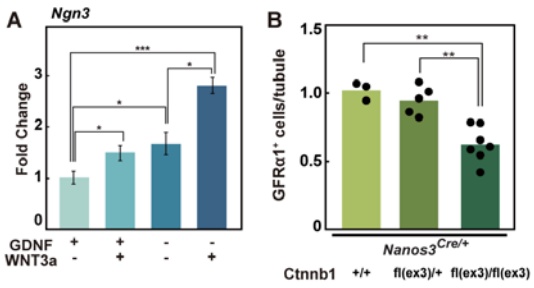

Figure 2. Wnt/β-catenin signal promotes the differentiation of GFRα1$^+$ spermatogonia *in vitro* and *in vivo*. (A) Expression of *Ngn3* mRNA in GS cells in the presence or absence of GDNF or WNT3a. GS cells were stimulated by the indicated conditions and analyzed by RT-qPCR for the *Ngn3* mRNA level. (B) Average numbers of GFRα1$^+$ cells per tubule, observed in mice with indicated genotypes. Number of GFRα1$^+$ cells was reduced in *Nanos3*$^{Cre/+}$;*Ctnnb1* $^{fl(ex3)/fl(ex3)}$ *mice*, in which β-catenin was activated in a germ cell specific manner. Modified from Tokue et al., Stem Cell Reports (2017),

2-2. SHISA6, a cell-autonomous Wnt inhibitor, is expressed in a subset of GFRα1$^+$ cells

We found that *Wnt6* is expressed in Sertoli cells, a supporting somatic cell type, in a spatially ubiquitous manner, suggesting that WNT6 is participating in the differentiation-promotion in seminiferous tubules, and that GFRα1$^+$ cells are uniformly exposed to WNT6 signals. These raised a next question: How do stem cells persist without exhaustion if they are equally bathed with Wnt ligand(s)?

To address this issue, we hypothesized that a Wnt inhibitor(s) may confer resistance to Wnt/β-catenin signaling in some fraction of GFRα1$^+$ cells. In our microarray data, we found *Shisa6* an interesting candidate, because *Shisa6* was highly enriched in the GFRα1$^+$ fraction and some of the other *Shisa* family members have been reported as Wnt inhibitors. Experiments using *Xenopus laevis* embryos and luciferase assays in HEK293T cells showed that SHISA6 is a novel Wnt inhibitor that acts autonomously (Figure 3A). Moreover, *Shisa6* expression was restricted in about 30% of the GFRα1$^+$ population *in vivo* (Figure 3B).

2-3. SHISA6$^+$ spermatogonia show stem cell characters and contribute to maintenance of GFRα1$^+$ cells

We found that, although *Shisa6* KO or stabilized *β-catenin* heterozygous mutants show no apparent phenotype in the pool of GFRα1$^+$ cells, synthetic heterozygotes for these alleles showed a reduced GFRα1$^+$ cell pool and spermatogenesis defects. Thus, SHISA6 plays a role in the maintenance of the stem cell pool by suppressing Wnt/β-catenin signaling in a cell-autonomous manner (Figure 4A).

We then addressed the behavior of SHISA6$^+$ cells conjectured from that of *T* (*Brachyury*)$^+$ cells, which were found to largely overlap with SHISA6$^+$ cells. A pulse-label and chase experiment showed that T$^+$ cells have the ability to continually produce progeny differentiating to sperm for at least 6 months. Together, these results suggest that T$^+$ (and probably SHISA6$^+$) cells have stem cell-related characteristics (Figure 4B)

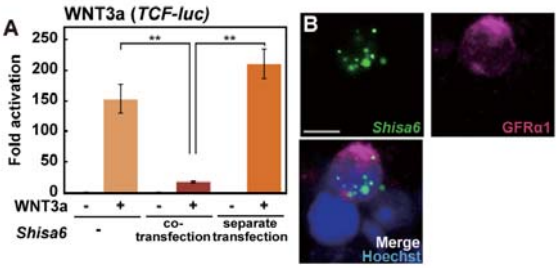

Figure 3. Function and expression of SHISA6 (A) Cell-autonomous inhibition of Wnt/β-catenin signaling by SHISA6. HEK293T cells were transfected with a Wnt reporter luciferase construct and a *Shisa6* expression plasmid either simultaneously (co-transfection), or separately (transfected cells were mixed afterword), followed by stimulation of Wnt/β-catenin signal. The Wnt-dependent Luciferase activity was suppressed only when Shisa6 was co-transfected. (B) Representative image of dissociated testicular cells of adult mice doubly stained for *Shisa6* by FISH and for GFR (B) Representative image. Note the overlapped expression of *Shisa6* and GFRsa is modified from Tokue et al., Stem Cell Reports (2017),

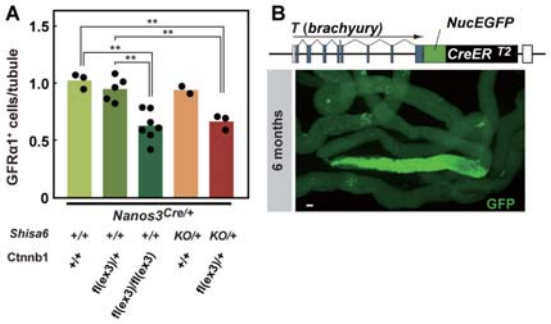

Figure 4. SHISA6 and spermatogenic stem cells (A) SHISA6 maintains the GFR S+ cell pool through inhibiting the Wnt/l pool through i. Average number of GFRve+ cells per tubule section in testes with the indicated genotypes, based on double IF for GFR microtesticular sections. (B) (Upper) A schematic of the structure of the *T*$^{hEGFP\text{-}CreERT2}$ allele, which enables pulse-labeling of T+ cells using CreER^{T2}. (Lower) A result of pulse-labelling of T+ cells with 6 months of chase. Note that GFP (the lineage reporter) positive cells form a prominent patch in a particular segment of seminiferous tubules. Modified from Tokue et al., Stem Cell Reports (2017),

2-4. SHISA6 protects the stem cells from differentiation promoting Wnt/β-catenin signaling: A proposal of a generic mechanism of stem cell regulation in facultative niches

To conclude, we propose a generic mechanism underlying the heterogeneous stem cell fates in facultative niche environments. Different levels of cell-autonomous inhibitor (SHISA6, in this case) may confer heterogeneous resistance to uniformly distributed extracellular signaling that promotes differentiation (such as WNTs). Here, stem cells with higher levels of inhibitors would remain in the undifferentiated cell pool with higher probabilities, while those with lower levels of inhibitors are more inclined to differentiate (Figure 5).

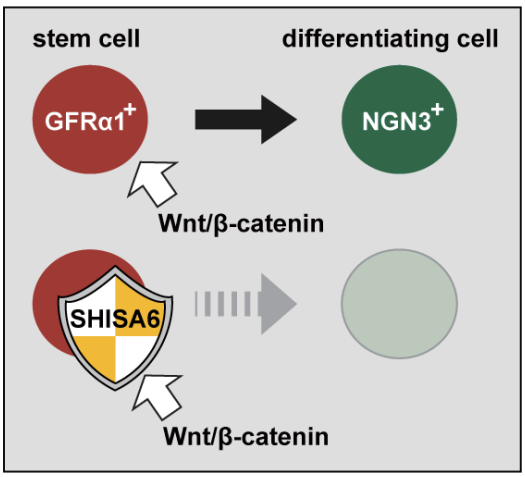

Figure 5. A conceptual diagram of differentiation promotion by Wnt/β-catenin signaling to become Ngn3+ and its cell-autonomous protection by SHISA6 in a subset of GFRd + cells. The heterogeneous expression of SHISA6 may confer different levels of differentiation probability in an open niche environment in which differentiation-promoting Wnt ligand appears to distribute uniformly. Modified from Tokue et al., Stem Cell Reports (2017),

Publication List:

〔Original Paper〕

- Tokue, M., Ikami, K., Mizuno, S., Takagi, C., Miyagi, A., Takada, R., Noda, C., Kitadate, Y., Hara, K., Mizuguchi, H., Sato, T., Taketo, M.M., Sugiyama, F., Ogawa, T., Kobayashi, S., Ueno, N., Takahashi, S., Takada, S., and Yoshida, S. (2017). SHISA6 confers resistance to differentiation-promoting Wnt/beta-catenin signaling in mouse spermatogenic stem cells. Stem Cell Rep. *8*, 561-575.

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The scope of our interests encompasses underlying mechanisms for the development of the vertebrate central nervous system (CNS) and various functions of the mature brain, including body fluid homeostasis, blood pressure control, food intake control, learning and memory.

I. Mechanisms for neural circuit formation

Adenomatous polyposis coli 2 (APC2) is preferentially expressed in the nervous system from early developmental stages through to adulthood. APC2 is distributed along actin fibers as well as microtubules in neurons. Our investigation suggests that APC2 is involved in the signaling pathway from membrane receptors for extracellular guidance factors to the intracellular migration machinery.

Sotos syndrome (OMIM #117550) is characterized by intellectual disability and a combination of typical facial features and large head circumference. Sotos syndrome has been known to be caused by haploinsufficiency in the *NSD1* gene. Our knockdown experiments revealed that the expression of *APC2* in the nervous system was under the control of *NSD1*. Moreover, *Apc2*-knockout (KO) mice also showed Sotos syndrome-like abnormalities. We are now investigating the relationship between NSD1 and APC2 in more detail by examining *Nsd1*-KO mice.

II.Physiological roles of receptor-like protein tyrosine phosphatases

Protein tyrosine phosphorylation plays crucial roles in various biological events such as cellular proliferation, differentiation, survival, migration, and metabolism. Cellular tyrosine phosphorylation levels are governed by the opposing activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). The physiological functions and regulatory mechanisms of receptor-like PTPs (RPTPs) are not fully elucidated. We have been making efforts to reveal the functional roles of RPTPs, especially of the R3 and R5 subfamilies.

2-1 R3 RPTP subfamily

The R3 RPTP subfamily, which is comprised of PTPRB, PTPRH, PTPRJ, and PTPRO, play pivotal roles in the development of several tissues including the vascular and nervous systems. Eph receptor tyrosine kinases are known to play indispensable roles in the topographic central projection of retinal ganglion cell (RGC) axons. Using the chick retinotectal projection system, we previously showed that PTPRO controls the sensitivity of retinal axons to ephrins and thereby has a crucial role in the establishment of topographic projections. We also demonstrated that R3 RPTPs dephosphorylate Eph receptors. Among R3 RPTPs, PTPRJ and PTPRO were expressed in developing mouse RGCs. We are now investigating the relationship between roles of PTPRJ and PTPRO in the central projection of the retinal axons by using gene knockout mice.

Leptin, an adipocyte-derived hormone, is a critical factor controlling food intake: it strongly inhibits food intake through the regulation of neuronal activities in the hypothalamic arcuate nucleus (ARC). Most obese individuals have an increased food intake despite high circulating leptin levels, which is referred to as leptin resistance. However, the exact mechanisms underlying leptin resistance in obese patients have yet to be elucidated. We recently demonstrated that PTPRJ is a physiological enzyme attenuating insulin signaling *in vivo*. In *Ptprj*-KO mice, the activation of insulin receptor (IR) kinase and Akt (or protein kinase B) was enhanced, and glucose and insulin tolerance was improved. Furthermore, *Ptprj*-KO mice exhibited lower weight gain associated with lesser food intake compared with wild-type mice.

We have now found that PTPRJ is involved in the regulation of leptin signaling in the ARC through the dephosphorylation of JAK2, the primary tyrosine kinase in leptin signaling. In line with these results, leptin signaling is enhanced in *Ptprj*-KO mice. Diet-induced obesity up-regulated PTPRJ expression in the hypothalamus, and induction of leptin resistance was strongly attenuated in *Ptprj*-KO mice. Upon leptin administration, *Ptprj*-KO mice fed a high-fat/high-sucrose

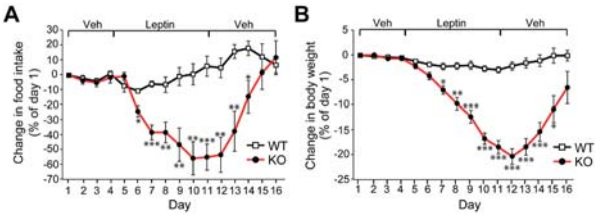

Figure 1. *Ptprj*-KO mice are protected from the development of diet-induced leptin resistance. (A) Daily change in food intake by and (B) Daily change in body weights of wild-type (WT) and *Ptprj*-KO mice fed high-fat/high-sucrose diet (HF/HSD for 14 weeks) upon daily administration of leptin. Leptin (500 ng) or vehicle was i.c.v. injected during the indicated period. ANOVA was used to detect significant differences at the same time point between the two groups (n = 8 each; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

diet showed significant reductions in food intake and body weight as compared with corresponding WT mice (Figure 1). Moreover, the overexpression of PTPRJ in the ARC in non-obese mice with a viral vector induced significant leptin resistance. Overall, our results indicated that PTPRJ plays critical roles in the development of leptin resistance *in vivo*.

2-2 R5 RPTP subfamily

PTPRZ is the most abundant RPTP in oligodendrocyte precursor cells (OPCs), which are the principal source of myelinating oligodendrocytes. Three PTPRZ isoforms are generated by alternative splicing from a single gene: two transmembrane isoforms, PTPRZ-A and PTPRZ-B, and one secretory isoform, PTPRZ-S (or phosphacan). All isoforms are heavily modified with chondroitin sulfate (CS) chains, and identified as chondroitin sulfate proteoglycans (CSPGs) in the CNS. The CS moiety on their extracellular domain of PTPRZ is essential for achieving high-affinity binding sites for the endogenous ligands such as pleiotrophin (PTN). We have proposed that PTPRZ is a new molecular target for drug development in glioblastoma and demyelinating diseases such as multiple sclerosis (MS).

CSPGs enriched in demyelinating plaques are known to impair remyelination by inhibiting the migration and differentiation of OPCs in MS patients. This year, we revealed that protamine (PRM) effectively neutralizes the inhibitory activities of CSPGs, thereby enhancing OPC differentiation and (re)myelination in mice. Cell-based assays revealed that PRM exerted masking effects on extracellular CSPGs, and also acted as a ligand mimetic for PTPRZ-A/B, thereby improving oligodendrocyte differentiation even under unfavorable differentiation conditions with CSPGs (Figure 2). Intranasal administration of PRM accelerated myelination in the developing mouse brain, and its intracerebroventricular administration stimulated remyelination after cuprizone-induced demyelination.

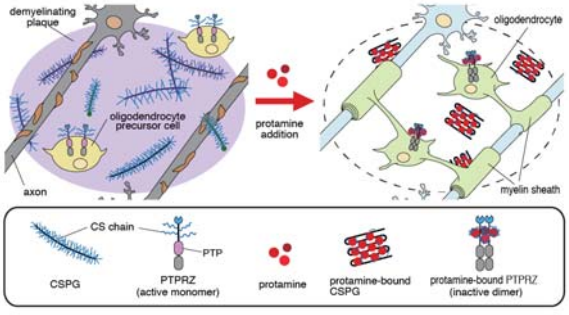

Figure 2. A possible regeneration model: Protamine may neutralize CSPG-mediated inhibition of migration of OPCs into demyelinating plaques and differentiation of OPCs to oligodendrocytes (see text).

A recent study on primary human glioblastomas suggested a close association between PTPRZ1 (human PTPRZ) expression and cancer stemness. However, the functional roles of PTPRZ in glioma stem cells have remained unclear. As was expected, sphere-forming cells from separated rat C6 and human U251 glioblastoma cell lines showed high expression of PTPRZ-B, the short receptor isoform of PTPRZ. Stable *PTPRZ* knockdown altered the expression levels of stem cell transcription factors such as SOX2, OLIG2, and POU3F2, along with decreased sphere-forming abilities of these cells.

We identified an allosteric PTPRZ inhibitor, NAZ2329 (Figure 3A) for the first time. This compound efficiently reduced the expression of SOX2 in C6 and U251 cells, and abrogated the sphere-forming abilities of these cells. Furthermore, tumor growth in the C6 xenograft mouse model was significantly slower with the co-treatment of NAZ2329 with temozolomide, an alkylating agent, than with the individual treatments (Figure 3B). These results indicate that PTPRZ is a promising molecular target for differentiation-inducing therapy of malignant gliomas.

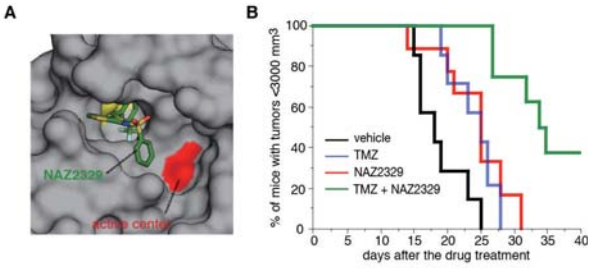

Figure 3. Identification of a new allosteric inhibitor, NAZ2329. **A**, X-ray structure of PTPRZ1-D1 complexed with NAZ2329. Cys1933 at the active site and Val-1911 at the bottom of the allosteric pocket are indicated in red and yellow, respectively. **B**, Antitumor effect of NAZ2329. Nude mice subcutaneously implanted with C6 glioblastoma cells were treated with DMSO, NAZ2329, temozolomide (TMZ), or the combination of NAZ2329 with TMZ until the humane endpoint (>3,000 mm^3 tumor size or 40 days after the treatment). Kaplan-Meier analysis of the four treatment groups.

III. Brain systems for body-fluid homeostasis

We have shown that Na_x, which structurally resembles voltage-gated sodium channels ($Na_v1.1$–1.9), is the brain $[Na^+]$ sensor to detect increases in $[Na^+]$ in body fluids. Na_x is preferentially expressed in specific glial cells of sensory circumventricular organs (sCVOs) including the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT). We have reported that Na_x signals in these brain regions deficient in a blood-brain barrier are involved in the control of water and salt intake.

3-1 Thirst control by Na_x and TRPV4

We previously demonstrated that Na_x signals in Na_x-positive glial cells leads to the activation of TRPV4-positive neurons by using epoxyeicosatrienoic acids as gliotransmitters in sCVOs to stimulate water intake. Also, we suggested that another $[Na^+]$-dependent signal is needed besides the Na_x-TRPV4 signal to explain the whole amount of water intake. In order to identify the sensor molecule which generates the unknown $[Na^+]$-dependent signal, we performed RNA-seq analysis of sCVOs and identified several candidates. We are now examining the functional roles of these candidates in water intake.

3-2 Thirst and salt-appetite control by angiotensin II, $[Na^+]$, and cholecystokinin

Angiotensin II (Ang II) is known to drive both thirst and salt appetite, and Ang II levels in blood are increased under both water- and salt-depleted conditions. However, we selec-

tively feel a thirst or salt appetite dependent on body fluid conditions. The brain mechanisms by which we properly take in water or salt have not been fully elucidated. Our local deletion experiments of *AT1a*, an Ang II receptor gene, indicated that AT1a signals in the SFO are involved in both water and salt intakes, whereas those in the OVLT are only involved in water intake. AT1a-positive neurons in the SFO project to several nuclei, including the OVLT and ventral part of the bed nucleus of the stria terminalis (vBNST). By using optogenetics, we demonstrated that thirst and salt appetite are driven by distinct groups of AT1a-positive neurons in the SFO: Neurons projecting to the OVLT control water intake, while those projecting to the vBNST control salt intake. We named these two different driver neurons 'water neurons' and 'salt neurons', respectively (Figure 4).

'Water neurons' were suppressed under Na-depleted conditions through cholecystokinin (CCK)-mediated activation of GABA(γ-aminobutyric acid)ergic neurons. As was expected, CCK levels in the SFO were increased under Na-depleted (water-satiated) conditions. The control mechanism of CCK secretion in the SFO remains to be elucidated. We had reported that Na_x-KO mice do not stop salt intake even under water-depleted conditions. We herein revealed that 'salt neurons' were suppressed under the water-depleted conditions through activation of another population of GABAergic neurons by Na_x signals. These studies thus provided new insights into the central mechanisms by which body fluid conditions control thirst and salt appetite. On the other hand, it is also known that aldosterone enhances salt appetite besides Ang II. Our next goal is integration mechanisms of salt-appetite signals from 'salt neurons' and 'aldosterone-sensitive neurons' in the vBNST.

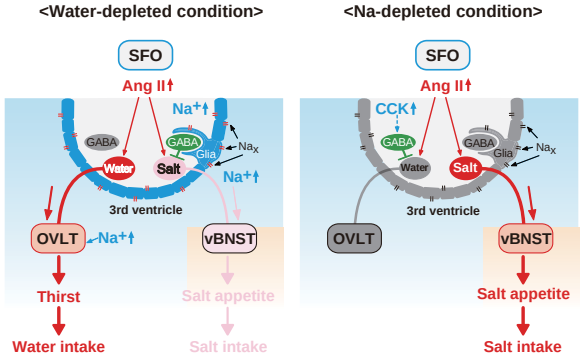

Figure 4. A schematic overview of control mechanisms for thirst and salt appetite in the SFO. Under the water-depleted condition (left), [Ang II] and [Na^+], but not [CCK], increase in the SFO, and "water neurons" innervating the OVLT are selectively activated. Under the Na-depleted condition (right), [Ang II] and [CCK], but not [Na^+], increase, and "salt neurons" innervating the vBNST are selectively activated. These two inhibitory systems by distinct GABAergic neurons are responsible for the specific activation of "water neurons" or "salt neurons" dependent on the body-fluid conditions. On the other hand, both water and Na intakes are simultaneously stimulated under the water- and Na-depleted condition, because the both neurons are active by Ang II.

Publication List:

〔Original papers〕

- Fujikawa, A., Chow, J.P.H., Matsumoto, M., Suzuki, R., Kuboyama, K., Yamamoto, N., and Noda, M. (2017). Identification of novel splicing variants of protein tyrosine phosphatase receptor type Z. J. Biochem. *162*, 381-390.
- Fujikawa, A., Sugawara, H., Tanaka, T., Matsumoto, M., Kuboyama, K., Suzuki, R., Tanga, N., Ogata, A., Masumura, M., and Noda, M. (2017). Targeting PTPRZ inhibits stem cell-like properties and tumorigenicity in glioblastoma cells. Sci. Rep. *7*, 5609.
- Hiyama, T.Y., Utsunomiya, A.N., Matsumoto, M., Fujikawa, A., Lin, C.-H., Hara, K., Kagawa, R., Okada, S., Kobayashi, M., Ishikawa, M., Anzo, M., Cho, H., Takayasu, S., Nigawara, T., Daimon, M., Sato, T., Terui, K., Ito, E., and Noda, M. (2017). Adipsic hypernatremia without hypothalamic lesions accompanied by autoantibodies to subfornical organ. Brain Pathol. *27*, 323-331.
- Kuboyama, K., Tanga, N., Suzuki, R., Fujikawa, A., and Noda, M. (2017). Protamine neutralizes chondroitin sulfate proteoglycan-mediated inhibition of oligodendrocyte differentiation. PLoS ONE *12*, e0189164.
- Matsuda, T., Hiyama, T.Y., Niimura, F., Matsusaka, T., Fukamizu, A., Kobayashi, K., Kobayashi, K., and Noda, M. (2017). Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. Nat. Neurosci. *20*, 230-241.
- Nakayama, K., Ohashi, R., Shinoda, Y., Yamazaki, M., Abe, M., Fujikawa, A., Shigenobu, S., Futatsugi, A., Noda, M., Mikoshiba, K., Furuichi, T., Sakimura, K., and Shiina, N. (2017). RNG105/caprin1, an RNA granule protein for dendritic mRNA localization, is essential for long-term memory formation. eLife *6*, e29677.
- Shintani, T., Higashi, S., Suzuki, R., Takeuchi, Y., Ikaga, R., Yamazaki, T., Kobayashi, K., and Noda, M. (2017). PTPRJ inhibits leptin signaling, and induction of PTPRJ in the hypothalamus is a cause of the development of leptin resistance. Sci. Rep. *7*, 11627.
- Utsunomiya, A.N., Okada, S., Hiyama, T.Y., Noda, M., and Kobayashi, M. (2017). Clinical characteristic features of adipsic hypernatremia patients wit subfornical organ-targeting antibody. Clin. Pediatr. Endocrinol. *26*, 197-205.

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The vertebrate central nervous system (CNS) contains many different types of neurons that form at distinct characteristic positions and develop specific axonal connections and functions. This complexity has made it difficult to perform detailed functional analysis of neuronal circuits: in particular, it was very difficult to reproducibly identify cell types during investigation. For the past 15 years, however, molecular genetic studies have strongly suggested that the expression of transcription factors in the developing CNS helps determine the morphological and functional properties of neurons. This has opened up the possibility that researchers can use these transcription factors as markers to identify cell types in the CNS. Transgenic animals that express fluorescent protein in specific subsets of neurons are particularly powerful tools to study functions of the corresponding neurons in the neuronal circuits.

To fully exploit the methodology described above, we use larval zebrafish as experimental animals. The biggest advantage of this system is that larval zebrafish are almost completely transparent. This allows us to utilize many optical techniques, including morphological/functional imaging and optogenetics. We can also perform targeted in vivo electrophysiological recordings with relative ease in this transparent model. An additional advantage of zebrafish is that their CNS is much simpler than mammals. This enables us to perform detailed functional analysis of neuronal circuits at a single cell resolution. Our hope is to reveal operational principles of vertebrate CNS by using this simple system.

We have been focusing on studying neuronal circuits that control locomotion. Much of the control of locomotor movements is accomplished by neuronal circuitry located in the spinal cord. Therefore, the focus of our studies has been spinal neuronal circuits in larval zebrafish.

In addition to zebrafish, we have also started to use medaka as an experimental animal. Medaka has many advantages similar to zebrafish. Because NIBB is the main center of the Medaka National Bioresource Project, we are in a perfect place to conduct experiments using medaka. To begin with, we explored whether knock-in fish could be efficiently generated using the CRISPR/Cas9 system.

I. Generation of Transgenic zebrafish

We have been generating transgenic zebrafish that express fluorescent proteins (GFP or RFP), Gal4, or Cre in specific classes of neurons in CNS by using promoter/enhancer of genes that are known to be expressed in subsets of neurons. Most of the genes we used are transcription factors expressed in subsets of neurons in the developing CNS. We also used those genes whose expressions are tightly related to neurotransmitter properties of neurons (i,e, vesicular glutamate transporter).

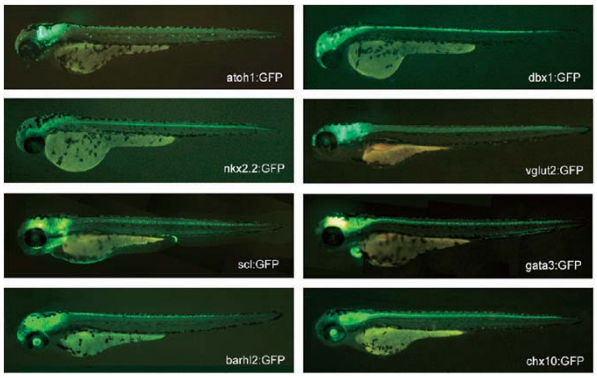

Figure 1. Examples of transgenic fish expressing GFP in specific classes of neurons.

In our early studies, we used a BAC-based transgenic technique for the generation of transgenic fish. In 2014, we succeeded in establishing a reliable knock-in method by utilizing the CRISPR-Cas9 system. The method we developed is highly efficient, such that nearly one-third of the raised animals become transgenic founders. Thus far, we have established more than 20 knock-in transgenic fish. The method greatly facilitates our functional analysis on neuronal circuits.

II. Neuronal circuits that control rhythmic pectoral fin movements.

Limbed vertebrates exhibit coordinated rhythmic movements of forelimbs and hindlimbs during locomotion. Neuronal circuits that control rhythmic limb movements in mammals have been investigated for decades, but our knowledge is still limited because of the complexity of their limb. Rhythmic movements of pectoral fins during swimming in larval zebrafish is an attractive model (Figure 2). Pectoral fins of larval zebrafish show left and right alternated rhythmic movements, and they are actuated only by two types of muscles, i.e., abductor (Ab) and adductor (Ad) (Figures 3). Due to the simplicity of pectoral fins, we expect that we well be able to characterize neuronal circuits that control rhythmic pectoral fin more deeply.

We performed electrophysiological recordings of Ab motoneurons (MNs) and Ad MNs during fictive swimming. Both Ab MNs and Ad MNs show rhythmic spiking activitie (Figure 4). Activities of Ab MNs and Ad MNs on the same side essentially alternated. Voltage clamp recordings showed that both Ab MNs and Ad MNs received alternating excitatory and inhibitory inputs in a swimming cycle. Excitations mainly occurred in their preferential firing phase, and inhi-

bition mainly occurred for the rest of the period. To obtain insights into the source of these inputs, we are now investigating timings of spiking activities in possible premotor interneurons.

Figure 2. Rhythmic movements of the pectoral fin (arrow) during swimming in larval zebrafish.

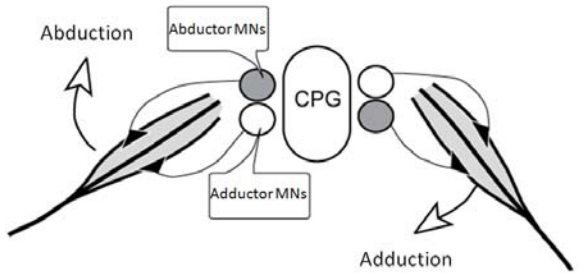

Figure 3. Schematic of rhythmic movements of pectoral fins during swimming. CPG, Central Pattern Generator.

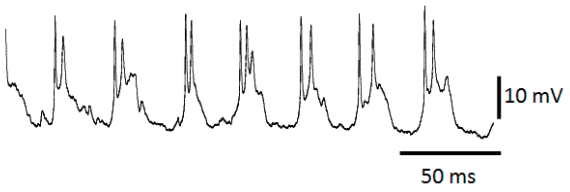

Figure 4. Rhythmic firings of an abductor motoneuron during swimming.

III. Functional analysis of En1-positive neurons for axial movements during swimming.

Inhibitions play important roles for shaping motor outputs during locomotion. In the spinal cord of larval zebrafish, there are mainly two types of inhibitory neuron: commissural inhibitory neurons and ipsilaterally-projecting inhibitory neurons. The role of the former (commissural inhibitory neurons) is easy to understand: they are likely to play important roles for ensuring antagonistic movements of the left and the right side of body. The role of the latter (ipsilaterally-projecting inhibitory neurons) is less clear.

En1-positive neurons constitute major components of ipsilaterally-projecting inhibitory neurons in the spinal cord. To investigate the function of En1-positive neurons, we have genetically-ablated En1-positive neurons by using the Cre-loxP system (Figure 5A). In the resultant larvae, the cycle period for the rhythmic bending of the body was prolonged (Figure 5B), indicating that swimming speed was slowed down. The results show that En1-positive neurons play an important role for controlling locomotion speed.

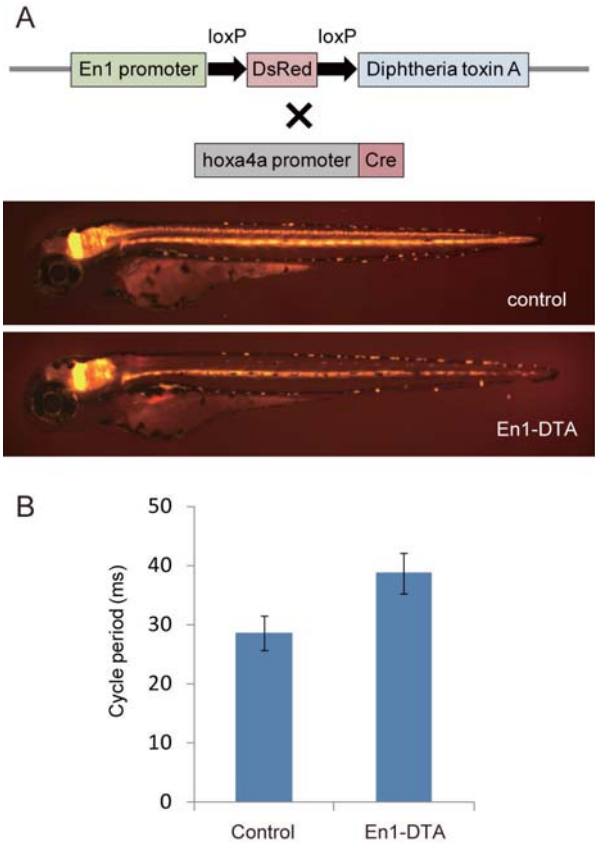

Figure 5. Spinal En1 neurons play an important role for controlling swimming speed. A, Ablation of spinal En1 neurons by using the Cre-loxP system. DTA, diphtheria-toxin A. B, Cycle period of the motor bursts during regular swimming in control and En1-DTA larvae.

IV. Axially-confined in vivo single-cell labeling by primed conversion using blue and red lasers with conventional confocal microscopes

Green-to-red photoconvertible fluorescent proteins (PCFPs), such as Kaede or Dendra2 are powerful tools to highlight a small number of cells in a globally fluorescent cellular context. However, perfect single-cell labeling in tightly packed tissues such as those that make up the nervous system has been difficult because the traditional method for efficient photoconversion requires the use of violet/UV single-photon excitation (i.e., 405 nm), which is not confined to the axial dimension. Under conditions where many cells express PCFPs in a three-dimensional manner, cells that are located above and below the target cells undergo photoconversion when exposed to a single-photon converting beam.

Recently, Dendra2 has been found to undergo efficient photoconversion by a new method termed primed conversion that employs dual wave-length illumination with blue and red/near-infrared light. By modifying a confocal laser-scanning microscope (CLSM) such that two laser beams only meet at the focal plane, confined photoconversion at the axial dimension has been achieved. The necessity of this custom modification to the CLSM, however, has precluded the widespread use of this method.

We investigated whether spatially-restricted primed

conversion could be achieved with CLSM without any hardware modifications. We found that the primed conversion of Dendra2 using a conventional CLSM with two visible lasers (473 nm and 635 nm) and a high NA objective lens (NA, 1.30) resulted in a dramatic restriction of photoconversion volume: half-width half-maximum for the axial dimension was below 5 μm, which is comparable to the outcome of the custom method that employed the microscope modification. As a proof of this method's effectiveness, we utilized this technique in living zebrafish embryos and succeeded in revealing the complex anatomy of individual neurons packed between neighboring cells. Because unmodified CLSMs are widely available, this method can be widely applicable for labeling cells with single-cell resolution.

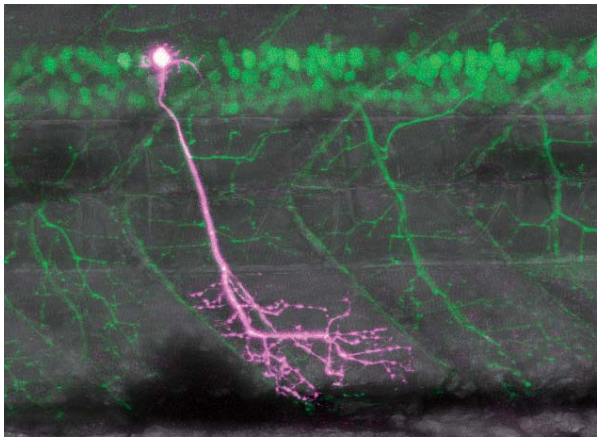

Figure 6. Primed conversion of Dendra2 reveals the fine morphology of a single motoneuron in a larval zebrafish

V. Highly efficient generation of knock-in transgenic medaka by CRISPR/Cas9-mediated genome engineering

As mentioned above, we have established an efficient (~30%) knock-in system via non-homologous end joining (NHEJ) using the CRISPR/Cas9 system in zebrafish. If the same technique were applicable in medaka, it would greatly expand the usefulness of this model organism. This point, however, has not yet been addressed.

We report the highly efficient generation of knock-in transgenic medaka via non-homologous end joining (NHEJ). Donor plasmid containing a heat-shock promoter and a reporter gene was co-injected with a short guide RNA (sgRNA) targeted for genome digestion, an sgRNA targeted for donor plasmid digestion, and Cas9 mRNA. In approximately 25% of injected embryos, broad transgene expression in the expression domain of a target gene was observed. By raising these animals, we succeeded in establishing stable knock-in transgenic fish with several different constructs for five genetic loci. The efficiencies of obtaining transgenic founders among the raised animals exceeded 50% for all five loci. Further, we show that the method is useful for obtaining mutant alleles. In the experiments where transgene integrations were targeted between the transcription start site and the initiation methionine, the resultant transgenic fish became mutant alleles.

With its simplicity, design flexibility, and high efficiency, we propose that CRISPR/Cas9-mediated knock-in via NHEJ will become a standard method for the generation of transgenic and mutant medaka. (Watakabe, I. et al. Zool. Lett. in press)

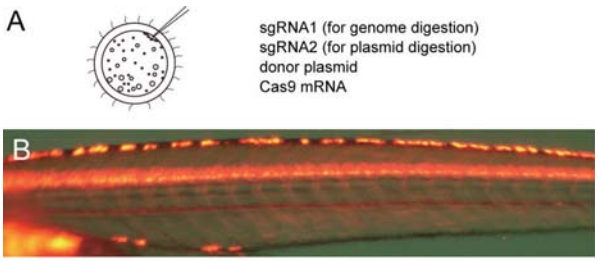

Figure 7. (A) For the generation of knock-in transgenic fish, sgRNA1 (for genome digestion), sgRNA2 (for plasmid digestion), donor plasmid with a bait sequence, and Cas9 mRNA are co-injected into one-cell-stage medaka embryos. (B) An example of transgenic medaka in which RFP was knocked-in into the vacht gene. As with the endogenous gene, RFP is expressed in motoneurons.

Publication List:

〔Original Papers〕

- Ratanayotha, A., Kawai, T., Higashijima, S., and Okamura, Y. (2017). Molecular and functional characterization of the voltage-gated proton channel in zebrafish neutrophils. Physiol. Rep. *15*, e13345.
- Taniguchi, A., Kimura, Y., Mori, I., Nonaka, S., and Higashijima, S. (2017). Axially-confined in vivo single-cell labeling by primed conversion using blue and red lasers with conventional confocal microscopes. Develop. Growth Differ. *59*, 741-748.

LABORATORY OF NEUROPHYSIOLOGY

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

"Why can we see?" This question is fundamental for a thorough understanding of vision-dependent animals, including human beings. In order to better understand the visual system of animals, we are researching animal behaviors through psychophysical and computational methods.

I. Psychophysical study of medaka fish

One of our major subjects is the psychophysical and computational study of medaka (*Oryzias latipes*, Matsunaga and Watanabe, 2010). Recently, we made progress in studies of prey-predator interaction using medaka and zooplankton. Visual motion cues are one of the most important factors for eliciting animal behavior, including predator-prey interactions in aquatic environments. To understand the elements of motion that cause such selective predation behavior, we used a virtual plankton system where the predation behavior in response to computer-generated prey was analyzed. Virtual prey models were programmed on a computer and presented to medaka, which served as predatory fish. Medaka exhibited predation behavior against several characteristic virtual plankton movements, particularly against a swimming pattern that could be characterized as pink noise motion. Analyzing prey-predator interactions via pink noise motion will be an interesting research field in the future (Matsunaga & Watanabe, 2012).

In recent years, we have made progress in studies of the schooling behaviors of medaka. Many fish species are known to live in groups. Visual cues have been shown to play a crucial role in the formation of shoals. Using biological motion stimuli, depicting a moving creature by means of just a few isolated points, we examined whether physical motion information is involved in the induction of shoaling behavior. We found that the presentation of virtual biological motion can prominently induce shoaling behavior. We have shown what aspects of this motion are critical in the induction of shoaling behavior. Motion and behavioral characteristics can be valuable in recognizing animal species, sex, and group members. Studies using biological motion stimuli will enhance our understanding of how non-human animals extract and process information which is vital for their survival (Nakayasu & Watanabe, 2014).

We have developed a novel method for behavior analysis using 3D computer graphics (Nakayasu et al., 2017). The fine control of various features of living fish have been difficult to achieve in studies of fish behavior. However, computer graphics allow us to manipulate morphological and motion cues systematically. Therefore, we have constructed 3D computer graphic animations of medaka based on tracking coordinate data and photo data obtained from real medaka. These virtual 3D models will allow us to represent medaka faithfully and to undertake a more detailed analysis of the properties of the visual stimuli that are critical for the induction of various behaviors. This experimental system was applied to studies on dynamic seasonal changes in color perception in medaka (Shimmura et al., 2017).

II. Psychophysical study of human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual illusions experienced by human beings (*Homo sapiens*). One recent focus of this debate is the flash-lag effect, in which a moving object is perceived to lead a flashed object when both objects are aligned in actual physical space. We developed a simple conceptual model (predictive coding, Figure 1) explaining the flash-lag effect (Watanabe et al., 2010). In recent years, we have made more developed novel visual illusions, such as the shelf-shadow illusion. This year, we began studying deep neural networks (DNNs) that represented the perceived rotational motion for illusion images that were not moving physically, much like human visual perception. (Figure 1). These models of DNNs will lead us in future work on perception science.

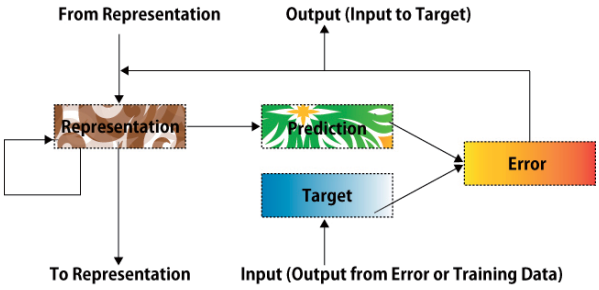

Figure 1. A schematic diagram of a predictive coding model of the brain. Illustration of information flow within a single layer is shown. Vertical arrows represent connections with other layers. Each layer consists of "Representation" neurons, which output a layer-specific "Prediction" at each time step, which is subtracted with "Target" to produce an error, which is then propagated laterally and vertically in the network. External data or a lower-layer error signal is input to "Target". In each layer, the input information is not processed directly, and the prediction error signal is processed.

Publication List:

〔Original papers〕

- Nakayasu, T., Yasugi, M., Shiraishi, S., Uchida, S., and Watanabe, E. (2017). Three-dimensional computer graphic animations for studying social approach behaviour in medaka fish: Effects of systematic manipulation of morphological and motion cues. PLoS ONE *12*, e0175059.
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I. Evolution of Complex Adaptive Characters

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

II. Spatiotemporal regulation of cell division axis as a grand plan of plant developmental evolution

Cell division axis has to be properly regulated during development in both metazoans and land plants. Genetic changes in the regulation of cell division axis lead to the development of multicellular organisms. Since land plants do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants should have different regulatory mechanisms. We aim to investigate the connecting factors between microtubules and GRAS transcription factors that regulate periclinal cell divisions in the moss *Physcomitrella patens*. In addition to identify the factors, the spatiotemporal regulatory mechanisms will be studies to understand the basis of body plan evolution with comparison to those in the flowering plant *Arabidopsis thaliana* and the green algae *Closterium peracerosum–strigosum-littorale*. This is a collabortion project between our division and Dr. Rumiko Kofuji in Kanazawa University, Dr. Hiroyuki Sekimoto in Japan Women's University, and Dr. Atsushi Mochizuki in RIKEN.

III. Evolution of Elaborated Cell Division Machinery: Spindle body

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a bipolar mitotic spindle, which is composed of microtubules. The centrosomes, which act as microtubule organizing centers, induce formation of the two poles in metazoan cells. In contrast, the cells of land plants and their sister group, zygnematales green algae, form the bipolar spindle in the absence of centrosomes. For understanding the mechanism of acentrosomal spindle formation, the steps of microtubule reorganization during spindle formation should be visualized. We collaborated with Prof. Tomomi Nemoto in Hokkaido University and developed a two-photon spinning disk confocal microscope, which enables 3-dimensional imaging of living cells with high temporal and spatial resolution. We also established a minispindle system, which involves a bipolar microtubule complex composed of an isolated chromosome and microtubules in tobacco cells. In collaboration with Dr. Daisuke Tamaoki (Toyama Univ.), analyses of microtubule behavior in the minispindle are in progress. Takashi Murata is a main researcher of this study.

IV. Evolution of Regeneration: Generic Regulatory Networks of Reprogramming of Differentiated Cells to Stem Cells

Both land plants and metazoa have the capacity to reprogram differentiated cells to stem cells. We found that the moss *Physcomitrella patens* Cold-Shock Domain Protein 1 (PpCSP1) regulates reprogramming of differentiated leaf cells to chloronema apical stem cells and shares conserved domains with the induced pluripotent stem cell factor Lin28 in mammals. PpCSP1 accumulates in the reprogramming cells and is maintained throughout the reprogramming process and in the resultant stem cells. Expression of *PpCSP1* is negatively regulated by its 3'-untranslated region (3'-UTR). Removal of the 3'-UTR stabilizes *PpCSP1* transcripts, results in accumulation of PpCSP1 protein, and enhances reprogramming. A quadruple deletion mutant of *PpCSP1* and three closely related *PpCSP* genes exhibits attenuated reprogramming indicating that the *PpCSP* genes function redundantly in cellular reprogramming. Taken together, these data demonstrate a positive role of PpCSP1

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

in reprogramming, which is similar to the function of mammalian Lin28 (Li et al. 2017).

We also found that histone chaperone HIRA proteins regulate stem cell formation through histone modification on *SQUAMOSA PROMOTER BINDING PROTEIN* (*PpSBP*) genes. Characterization of *HIRA*s and *PpSBPs* are in progress mainly by Yukiko Kabeya and Yosuke Tamada.

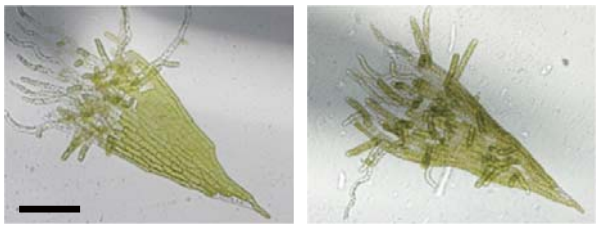

Figure 1. Cells facing the cut of dissected gametophore leaves were reprogrammed to be stem cells in wild type (left), while both edge and inner cells were reprogrammed with PpCSP1 accumulation (right).

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

Animal somatic cells can be reprogrammed to iPS cells by introducing four transcription factors, while such factors have not been identified in plants. We found a gene encoding a member of a plant-specific transcription factor, STEM CELL-INDUCING FACTOR 1 (STEMIN1) that was able to induce direct reprogramming of differentiated leaf cells into chloronema apical stem cells without wounding signals. To understand the role of STEMIN1 in reprogramming, we investigate STEMIN1-direct target genes identified by RNA-seq and ChIP-seq analyses. Masaki Ishikawa and Mio Morishita are this study's main researchers.

VI. Evolution of Regeneration: Stem cells laterally inhibit surrounding cells

Under certain circumstances differentiated cells can be reprogrammed to form stem cells in land plants, but only a portion of the cells reprogram successfully. A long-distance inhibitory signal from reprogrammed cells to surrounding cells has been reported in some ferns. We showed the existence of an anisotropic inhibitory signal to regulate stem cell formation in the moss *Physcomitrella patens*. When single cells were isolated from a gametophore leaf, over 90% of them were reprogrammed to stem cells with characteristic nuclear expansion. By contrast, when two adjacent cells were isolated, the nuclei of both cells expanded, but successful reprogramming of both cells occurred only in approximately one fifth of the pairs. When three aligned cells were isolated, the reprogramming rate of both edge cells decreased with a living middle cell but did not with a dead middle cell. Furthermore, unequal conversion into stem cells was more prominent in cell pairs aligned parallel to the proximal-distal leaf axis than in those perpendicular to the axis. This study gives an insight into the role of the inhibitory signal in development and evolution as well as efficient stem cell induction from differentiated cells (Sato et al. 2017). Liechi Zhang is investigating the factors involved in the lateral inhibition.

VII. Evolution of Regeneration: Other pathways

Nan Gu, a joint graduate student between Huazon Agricultural University and NIBB is interested in DNA damage and reprogramming, and is working with the mechanisms connecting DNA damage and reprogramming of differentiated cells to stem cells.

We found that INHIBITOR OF GROWTH (ING) proteins are involved in the stem cell formation of cut leaves. The ING proteins are known to regulate an apoptosis pathway in animals but plants do not have the corresponding pathway. Akihiro Imai, a former postdoc in this division and now an Assistant Professor in Hiroshima Institute of Technology is investigating the molecular function of ING as a collaboration work.

VIII. Evolution of Molecular Mechanisms of Plant Movement

The sensitive plant *Mimosa pudica* and the carnivorous plants sundew (*Drosera spatulata*) and Venus fly trap (*Dionaea muscipula*) have long attracted the interest of researchers due to their spectacular leaf movements in response to touch or other external stimuli. Although various aspects of the movement have been elucidated by physiological, cytological or biochemical approaches, the lack of genetic tools hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we sequenced and analyzed their genomes. Furthermore, we developed an efficient genetic transformation method for these plants and established a CRISPR/Cas9-mediated gene knock-out system in *M. pudica*. We isolated several candidate genes that may play roles in the seismonastic movement of *M. pudica* by comparing gene expression profiles between motor organs (pulvini) and non-motor organs and between extensor and flexor halves of pulvini. Functional analyses of these genes with the CRISPR/Cas9 system are in progress. The studies on *M. pudica* and carnivorous plants were conducted mainly by Hiroaki Mano and Hiraku Suda, respectively.

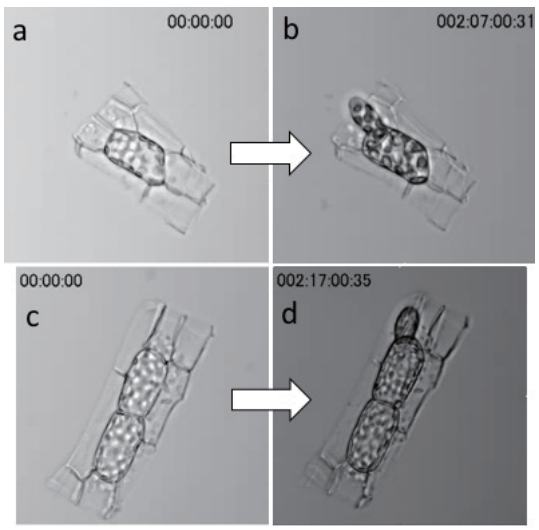

Figure 2. Reprogramming of isolated gametophore leaf cells of the moss *Physcomitrella patens*. A single cell (a, b) and two adjacent cells (c, d) were isolated by hand using a carbon knife.

IX. Evolution of Plant Development

To investigate evolution of novel complex traits, the following studies are ongoing with graduate students: Chiharu Kamida studies genes involved in movable tentacle development in the sundew *Drosera spatulata*. Shizuka Koshimizu is interested in the evolution of floral homeotic genes and investigates the function of MADS-box genes in the non-flowering plant *Physcomitrella patens*. The pseudanthium is a flower-like inflorescence, the molecular mechanisms of the development of which are unknown. Tomomi Sugaya succeeded in transferring the *FT* gene from *Arabidopsis thaliana* into the pseudanthium *Houttuynia cordata*. Furthermore, introduction of the *FT* gene successfully induced flowers. Ruan de Villiers investigates the evolution of signaling pathways of the phytohormone strigolactone in land plants.

X. Evolution of Digestive Enzymes and Pitcher Leaves in Carnivorous Plants

Carnivorous plants exploit animals as a nutritional source and have inspired long-standing questions about the origin and evolution of carnivory-related traits. To investigate the molecular bases of carnivory, we sequenced the genome of the heterophyllous pitcher plant *Cephalotus follicularis*, in which we succeeded in regulating the developmental switch between carnivorous pitcher and non-carnivorous flat leaves by using different temperatures. Transcriptome comparison of the two leaf types and gene repertoire analysis identified genetic changes associated with prey attraction, capture, digestion and nutrient absorption. Analysis of digestive fluid proteins from *C. follicularis* and three other carnivorous plants with independent carnivorous origins revealed repeated co-options of stress-responsive protein lineages coupled with convergent amino acid substitutions to acquire digestive physiology. These results imply constraints on the available routes to evolve plant carnivory.

To further investigate the molecular mechanisms to regulate flat and pitcher leaves, Gergo Palfalvi analyzes transcriptomes and chromatin modifications as a collaboration work with Dr. Kenji Fukushima in Colorado University.

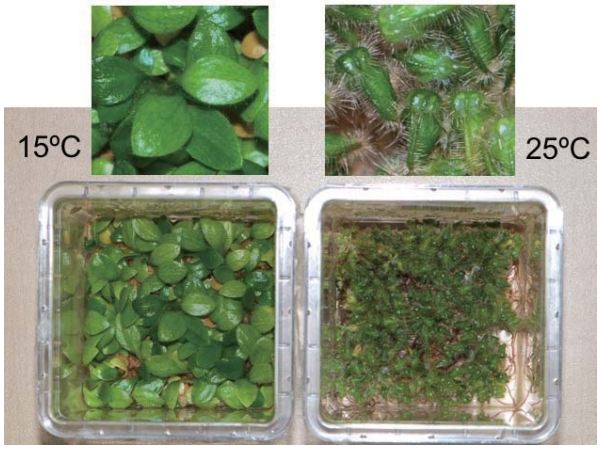

Figure 3. *Cephalotus follicularis* preferentially produces flat leaves at 15ºC (left) and pitcher leaves at 25ºC.

Publication List:

〔Original papers〕

- Fukushima, K., Fang, X., Alvarez-Ponce, D., Cai, H., Carretero-Paulet, L., Chen, C., Chang, T.-H., Farr, K.M., Fujita, T., Hiwatashi, Y., Hoshi, Y., Imai, T., Kasahara, M., Librado, P., Mao, L., Mori, H., Nishiyama, T., Nozawa, M., Pálfalvi, G., Pollard, S.T., Rozas, J., Sánchez-Gracia, A., Sankoff, D., Shibata, T.F., Shigenobu, S., Sumikawa, N., Uzawa, T., Xie, M., Zheng, C., Pollock, D.D., Albert, V.A., Li, S., and Hasebe, M. (2017). Genome of pitcher plant Cephalotus reveals genetic changes associated with carnivory. Nat. Ecol. Evol. 1, 0059.
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Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on earth. In this symbiosis, soil bacteria called rhizobia supply the host legumes with ammonia produced through bacterial nitrogen fixation. In return, host plants provide the rhizobia with their photosynthetic products. To accomplish this biotic interaction, leguminous plants develop nodules on their roots. On the other hand, more than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. Despite marked differences between the fungal and bacterial symbioses, common genes are required for both interactions. Using a model legume *Lotus japonicus*, we are trying to unveil the molecular mechanisms of both symbiotic systems.

I. AM symbiosis

1-1 Non-tandemly repeated heterogeneous rDNAs of arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF) are one of the most widespread symbionts of land plants. Our substantially improved reference genome assembly of a model AMF, *Rhizophagus irregularis* DAOM-181602 (total contigs = 210, contig N50 = 2.3Mbp, INSDC# = BDIQ01000001 -BDIQ01000210) facilitated discovery of repetitive elements with unusual characteristics. *R. irregularis* has only ten to eleven copies of the complete 45S rDNA (Figure 1A), whereas the general eukaryotic genome has tens to thousands of rDNA copies. *R. irregularis* rDNAs are highly heterogeneous and lack a tandem repeat structure (Figure 1A, B). These findings provide evidence for the hypothesis of concerted evolution that rDNA homogeneity depends on its tandem repeat structure. RNA-seq analysis confirmed that all rDNA variants are actively transcribed. Observed rDNA/rRNA polymorphism may modulate translation by using different ribosomes depending on biotic and abiotic interactions. The non-tandem repeat structure and intragenomic heterogeneity of AMF rDNA may facilitate adaptation to a broad host range despite lacking a sexual life cycle.

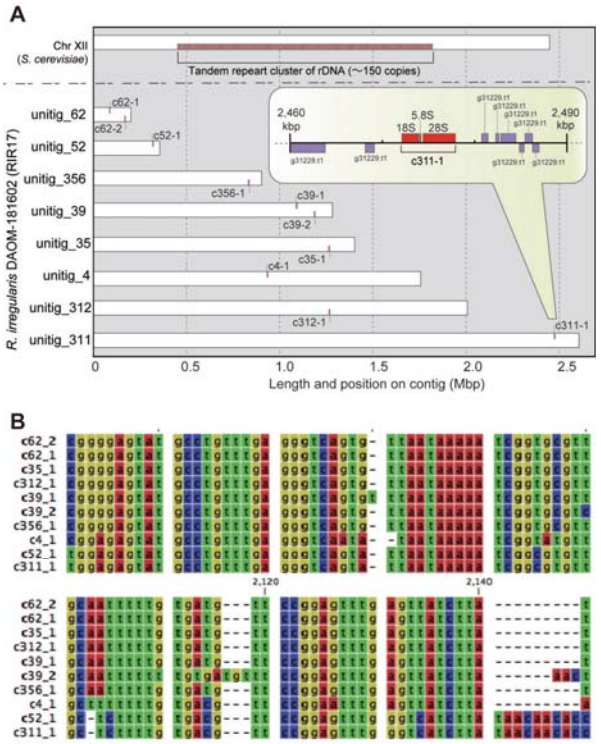

Figure 1. Ribosomal DNA genes of *Rhizophagus irregularis* DAOM-181602
A. Distribution of *R. irregularis* rDNA units in the genome. Each 48SrDNA unit is represented as a red box. For comparison, rDNA clusters on *Saccharomyces cerevisiae* chromosome XII is shown. Inset is a magnified view of a 48rDNA unit (c311-1) with nearby protein-encoding genes (purple boxes). Genes encoded on plus strand genome are depicted on the top side, and those encoded on minus strand are shown on the bottom side. B. Alignment of a heterogeneous region among the 48SrDNA paralogs. Partial sequences of Mafft-aligned 48SrDNAs (corresponding 2,049-2,136 bp positions on c62-1).

II. Root nodule symbiosis

2-1 *NITRATE UNRESPONSIVE SYMBIOSIS 1* mediates nitrate-induced control of root nodule symbiosis

Symbiotic nitrogen fixation in root nodules containing symbiotic rhizobia enables legumes to thrive under nitrogen-deficient environments. However, the symbiosis is known to be an energy consuming activity in which photosynthates are used as an energy source. Therefore, plants may cease the symbiosis if there is a sufficient nitrogen source available in their environment, thereby enabling plants to save the cost associated with nodulation.

Legumes control the number of nodules per root system through a mechanism called autoregulation of nodulation (AON), a systemic long-range signaling between roots and shoots. In addition to their hypernodulating-phenotypes, mutants involved in the AON retain nodule formation even

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

in the presence of a high nitrate concentration. Furthermore, production of CLE-RS2, a proposed root-derived signal in AON, is induced not only by rhizobial infection but also by nitrate application. These observations suggest that the mechanism for nitrate-induced control of nodulation shares common elements with the AON. In contrast, some findings suggest that fundamental knowledge of AON is insufficient to account for a regulatory mechanism, indicating that new factors await discovery.

We identified a novel *Lotus japonicus* mutant, *nitrate unresponsive symbiosis 1* (*nrsym1*) that formed mature nodules even in nitrate-sufficient conditions. In wild type, a high concentration of nitrate reduced infection thread number, nodule number, nodule size and nitrogen fixation activity. In contrast, these inhibitory effects were suppressed in *nrsym1* mutants. These data indicate that NRSYM1 mediates pleiotropic nitrate-induced control of root nodule symbiosis. In addition, analysis of the loss-of-function mutation of *CLE-RS2* and *HAR1*, encoding a receptor of CLE-RS2 in AON, indicate that AON may be involved in the regulation of nitrate-induced inhibition of nodule number, but rhizobial infection, nodule growth, and nitrogen fixation activity

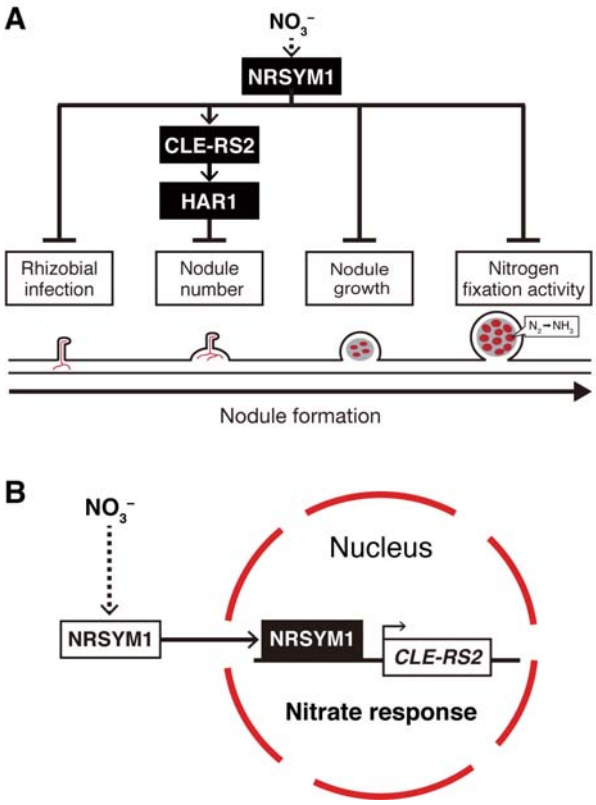

Figure 2. Model for the control of root nodule symbiosis in response to nitrate. (A) Sequential progress of nodulation is shown. In response to nitrate, NRSYM1 regulates pleiotropic phases of root nodule symbiosis, including rhizobial infection, nodule number, nodule growth, and nitrogen fixation activity. Whereas NRSYM1 activates the CLE-RS2>HAR1 signaling pathway leading to the negative regulation of nodule number, NRSYM1 is likely to use different downstream targets to achieve the regulation of other nitrate-affected processes. Red lines and red cells respectively indicate the infection threads and rhizobia-colonized cells. (B) A model for cellular-level NRSYM1 function. NRSYM1 activated by a nitrate-dependent nuclear retention mechanism regulates root nodule symbiosis by directly regulating nodulation-related genes such as *CLE-RS2*.

are controlled through a mechanism independent of AON (Figure 2A). *NRSYM1* encodes a NIN-LIKE PROTEIN transcription factor. The expression of *CLE-RS2* was strongly induced in wild type by nitrate treatment, but the induction levels were much lower in *nrsym1* roots. Furthermore, ChIP-qPCR analysis suggested that NRSYM1 can directly bind to the promoter regions of *CLE-RS2* in a nitrate-dependent manner. The expression of *NRSYM1* is not induced by nitrate, suggesting that post-translational regulation of NRSYM1 provides it with function. We then examined the subcellular localization of NRSYM1 by immunohistochemistry. Although NRSYM1 was barely detected in nuclei in nitrate-free conditions, the protein was predominantly localized in nuclei in the presence of nitrate. Our data indicate that NRSYM1 is activated by a nitrate-dependent nuclear retention mechanism and directly regulates the production of CLE-RS2, thereby triggering the negative regulation of nodule number through AON (Figure 1B).

2-2 *Lotus japonicus* ERN1 is essential for early processes of bacterial entry during nodulation

Bacterial entry into host tissues are essential processes to establish the symbiotic interaction between rhizobia and host legumes. Infection processes are initiated by adhesion of rhizobia on surfaces of root hairs and secretion of bacterial nodulation signaling molecules, Nod factors, which cause tip deformation of root hairs. Rhizobia entrapped by curling of the deformed root hair penetrate into inner tissue layers through infection threads (ITs), host-membranous and tubular structures developed from infection foci. Nod factor receptors and common symbiosis factors are necessary for symbiotic root hair responses. However, nodulation specific factors involved in rhizobial infection are largely unknown.

We conducted forward genetic screening to identify new host factors involved in bacterial entry processes, and isolated two allelic mutant lines, F29 and 1699-1, that exhibit defects in IT development, from M2 populations of EMS-treated and ion beam-mutagenized *L. japonicus* MG-20 seeds, respectively. The causative gene encoded a member of plant-specific APETALA2 (AP2) / ETHYLEN RESPONSE FACTOR (ERF) family TFs, namely ERF REQUIRED FOR NODULARION1 (ERN1). Total numbers of ITs in *ern1* null mutants (1699-1) were less than 1% of wild type plants. Even if ITs were developed on *ern1* roots, they were aberrantly short, and arrested in root hairs (Figure 3). Consequently, ramified ITs that are usually observed in wild type nodules were absent in *ern1* nodules. Furthermore, significant numbers of *ern1* root hairs were abnormally swollen, and exhibited a balloon-like shape only when inoculated with rhizobia. These results suggested that ERN1 is not necessary for Nod factor perception because root hairs were able to respond to inoculation, but is required for correct root hair responses besides developmental processes of ITs.

ERN1 expression was upregulated within 12 hours after inoculation. Spatial expression pattern analysis using a *ß-glucuronidase* reporter gene showed that *ERN1* was expressed in infected root hairs and in developing nodules. The expression pattern was consistent with the role of ERN1 in early processes of nodulation after Nod factor perception.

This expression in response to rhizobial infection required CYCLOPS, a TF acting in the common symbiosis pathway, suggesting that ERN1 is a factor downstream of the common symbiosis pathway. Phylogenetic analysis showed that ERN1 is conserved in leguminous and non-leguminous plants. ERN1 may contribute to the bacterial entry by recruiting its downstream genes required for physiological and cellular events widely conserved in plants.

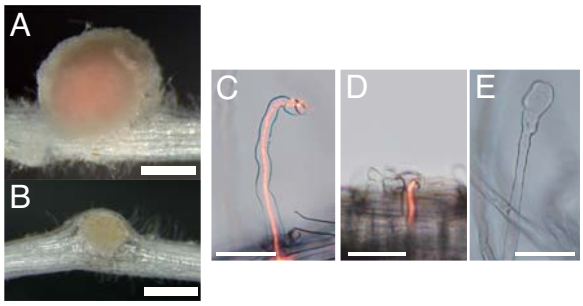

Figure 3. Root nodules formed in L. *japonicus* wild type (A) and *ern1* (1699-1) mutant roots (B). Root hair phenotypes of wild type (C), *ern1-5* (F29; D), and *ern1-6* (1699-1; E) mutants after inoculation with DsRed-labeled *Mesorhizobium loti*. Merged images of DsRed fluorescence and bright-field (C, D). Scale bars; 0.5 mm in (A, B), 50 μm in (C-E).

III. Spatial regularity control of phyllotaxis pattern generated by the mutual interaction between auxin and PIN1

Phyllotaxis, the arrangement of leaves on a plant stem, is well known because of its beautiful geometric configuration, which is derived from the constant spacing between leaf primordia. Phyllotaxis patterns are established by the mutual interaction between a diffusible plant hormone auxin and its efflux carrier PIN1, which cooperatively generate a regular pattern of auxin maxima, small regions with high auxin concentrations, leading to leaf primordia. However, the molecular mechanism of auxin maxima patterning is still largely unknown. To better understand how the phyllotaxis pattern is controlled, we investigated mathematical models based on the auxin–PIN1 interaction through linear stability analysis and numerical simulations, focusing on the spatial regularity control of auxin maxima.

As in previous reports, we first confirmed that this spatial regularity can be reproduced by a highly simplified and abstract model. However, this model lacks the extracellular region and is not appropriate for considering the molecular mechanism. Thus, we investigated how auxin maxima patterns are affected under more realistic conditions. We found that the spatial regularity is eliminated by introducing the extracellular region, even in the presence of direct diffusion between cells or between extracellular spaces, and this strongly suggests the existence of an unknown molecular mechanism. To unravel this mechanism, we assumed a diffusible molecule to verify various feedback interactions with auxin–PIN1 dynamics. We revealed that regular patterns can be restored by a diffusible molecule that mediates the signaling from auxin to PIN1 polarization. Furthermore, as in the one-dimensional case, similar results are observed in the two-dimensional space. These results provide a great insight into the theoretical and molecular basis for understanding the phyllotaxis pattern. Our theoretical analysis strongly predicts a diffusible molecule that is pivotal for the phyllotaxis pattern but is yet to be determined experimentally.

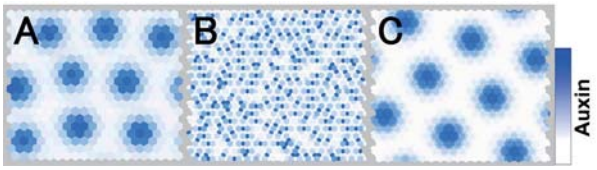

Figure 4. Examples of computer simulations in two-dimensional space. The regular spatial pattern of auxin maxima can be generated under simplified conditions in the absence of the extracellular region (A). However, this spatial regularity is completely disrupted by introducing the extracellular region (B), but can be restored by assuming a diffusible molecule that mutually interacts with auxin–PIN1 dynamics (C).

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〔Original papers〕

- Carotenuto, G., Chabaud, M., Miyata, K., Capozzi, M., Takeda, N., Kaku, H., Shibuya, N., Nakagawa, T., Barker, D., and Genre, A. (2017). The rice LysM receptor-like kinase *Os*CERK1 is required for the perception of short-chain chitin oligomers in arbuscular mycorrhizal signaling. New Phytologist *214*, 1440-1446.
- Ohtsu, M., Sato, Y., Kurihara, D., Suzaki, T., Kawaguchi, M., Maruyama, D., and Higashiyama, T. (2017). Spatiotemporal deep imaging of syncytium induced by the soybean cyst nematode *Heterodera glycines*. Protoplasma *254*, 2107-2115.
- Ohtsu, M., Kurihara, D., Sato, Y., Suzaki, T., Kawaguchi, M., Maruyama, D., and Higashiyama, T. (2017). Fluorescent labeling of the cyst nematode *Heterodera glycines* for deep tissue live imaging using two-photon microscopy. Cytologia *82*, 251-259.
- Yano, K., Aoki, S., Liu, M., Umehara, Y., Suganuma, N., Iwasaki, W., Sato, S., Soyano, T., Kouchi, H., and Kawaguchi, M. (2017). Function and evolution of a *Lotus japonicus* AP2/ERF family transcription factor that is required for development of infection threads. DNA Res. *24*, 193-203.
- Maeda, T., Kobayashi, Y., Kameoka, H., Okuma, N., Takeda, N., Yamaguchi, K., Bino, T., Shigenobu, S., and Kawaguchi, M. (2017) Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*. bioRxiv preprint first posted online Oct. 19, doi: https://doi.org/10.1101/205864.

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

- Yamaya-Ito, H., Shimoda, Y., Hakoyama, T., Sato, S., Kaneko, T., Hossain, MS., Shibata, S., Kawaguchi, M., Hayashi, M., Kouchi, H., and Umehara, Y. Loss-of-function of ASPARTIC PEPTIDASE NODULE-INDUCED 1 (APN1) in *Lotus japonicus* restricts efficient nitrogen-fixing symbiosis with specific *Mesorhizobium loti* strains. Plant J. 2017 Dec 2.

DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY

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The Division of Evolutionary Developmental Biology was started in June 2015. We focus on the evolutionary novelties acquired by insects through evolution, in order to elucidate the molecular and evolutionary mechanisms leading to the large variety of traits that they display. From this wealth of exciting traits, our lab currently focuses on promoting research into (1) the origin and diversification of insect wings, (2) wing color patterns and mimicry of ladybird beetles, and (3) acquisition and diversification of beetle horns.

I. Origin and diversification of insect wings

Of the various flying animals on the earth, insects have evolved a unique flight organ. Despite over two centuries of debate, the evolutionary origin of the insect wing is still an enigma. We try to approach this issue using evo-devo methods. In *Drosophila melanogaster*, the wing master gene *vestigial* (*vg*) and its interaction partner *scalloped* (*sd*) play pivotal roles in the formation of wing field identity. For this reason, these genes are ideal candidates for investigating wing origin and evolution.

One way to identify the structure from which insect wings first evolved is to explore the function of "wing" genes in ancestral wingless (apterygote) species. We chose the firebrat, *Thermobia domestica,* as a model (Figure 1A). *T. domestica* belongs to Thysanura, phylogenetically the closest extant relative of winged (pterygote) insects, making it ideal for elucidating wing origin. We cloned *vg* and *sd* orthologs from *T. domestica* (*Td-vg* and *Td-sd*). To examine the functions of these genes, we developed RNA interference (RNAi) based methods for *T. domestica*. We are currently testing for functional effects of altered transcription of each of these wing genes in the ancestrally wingless firebrats. In addition, we are performing comparative analyses of the function of these same genes in "primitively winged" (hemimetabolous) insects, to obtain additional clues relevant to understanding the origin and evolution of insect wings.

Interestingly, our previous work showed that *vg* expressing epidermal tissue forms lateral outgrowths in non-winged segments in a mealworm beetle (Ohde *et al*., 2013). From these facts, we hypothesize that ancestral lateral body wall outgrowths evolved into functional wings. To test this hypothesis, we are now comparing the role of *vg* and other "wing genes" between wings and lateral outgrowths in a basal winged insect, *Gryllus bimaculatus,* and non-winged insect, *Thermobia domestica* (Figure 1).

Figure 1. The firebrat, *Thermobia domestica* (A). the two-spotted cricket, *Gryllus bimaculatus* (B).

II. Wing color patterns and mimicry of ladybird beetles

Insects have evolved a tremendous range of diversity of wing color patterns which play various ecologically important roles such as intraspecific sexual signaling, mimesis, mimicry, and warning against predators. The molecular mechanisms responsible for generating such color patterns in most ladybird species, however, remain elusive. To investigate the developmental mechanisms of color pattern formation, we have been focusing on the multicolored Asian ladybird beetle, *Harmonia axyridis*, which has conspicuous and variable wing color patterns consisting of black and red pigments (Figure 2A). Vivid wing color patterns of ladybirds function as a warning signal to convey to predators that they are distasteful. At the same time, various other insect species are utilizing this ecological signal by mimicking the ladybirds' wing color patterns. Mimicry provides an exciting opportunity to study how independent lineages of insects have evolved convergent color patterns. To explore color pattern formation mechanisms in mimicry, we are focusing on the leaf beetle, *Argopistes coccinelliformis*, which has color patterns similar to *Harmonia*, and which is thought to be a Batesian mimicry of ladybird beetles (Figure 2B). To elucidate the molecular mechanisms underlying these wing color patterns, we established a technique for germline

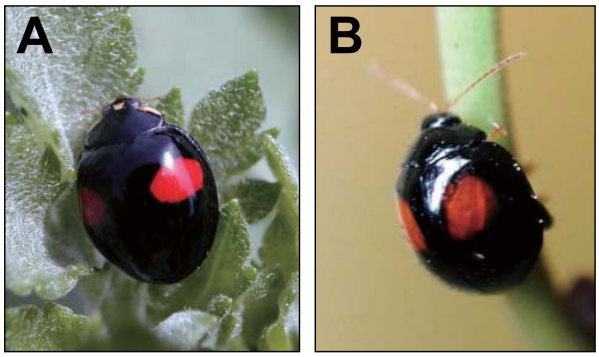

Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

transformation using a *piggyBac* vector and RNAi in the ladybirds.

Recently, we identified several key regulatory genes associated with the color pattern formation of ladybird beetles using next generation sequencing technologies (RNA-seq and *de novo* genome assembly), and an RNAi-based screening method that we established. Many of these genes are expressed in specific domains of wing color patterns, suggesting that regulatory shift, such as change in enhancer activity, at these gene loci may be crucial for evolution of wing color patterns in ladybirds. Now, we are trying to elucidate the evolutionary origin of color patterns in ladybirds focusing on regulatory shifts at these genetic loci. To tackle this issue, we are establishing genome-editing technologies using TALEN and CRISPR/Cas9, and have already established an efficient method of gene disruption. At present, we are establishing more complicated genome editing techniques such as genomic insertion, inversion and duplication to identify the crucial regulatory shift that may have driven evolution of wing color patterns in ladybird beetles.

In the future, we are planning to analyze how the similar wing color patterns of model and mimic are generated based on the knowledge obtained from *H. axyridis*, – for example, do they use conserved or divergent mechanisms?

III. Acquisition and diversification of beetle horns

Insects show a tremendous range of diversity in "horns", rigid body outgrowths that function as weapons. Horns are exciting for evo-devo studies because they have arisen multiple times *de novo,* as evolutionary "novelties". However, the molecular mechanisms involved in sexually dimorphic horn formation are still poorly understood. To investigate the developmental mechanisms of horn formation, we focus on the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns (Figure 3A). The male-specific horns of *T. dichotomus* are one of the best models to study how an extreme, sex-specific morphology is formed. We have developed a technique for larval RNAi in *T. dichotomus,* permitting us to rigorously and systematically test the functional roles of a large suite of candidate developmental genes, revealing for the first time the molecular mechanisms responsible for growth of male rhino beetle horns. In addition, we are employing a high throughput approach. To identify novel genes involved in the sexually dimorphic horn development in *T. dichotomus*, mRNA of the developing horn discs has been assessed by deep-sequencing transcriptome analysis (RNA-seq). We narrowed down the genes associated with horn formation to 40 genes, and performed RNAi-based knockdown screening to provide deep insights into where, when, and how the head and thoracic horns are formed during development. We successfully identified 11 transcription factors that contribute to horn formation. These 11 genes include larval head- and appendage-patterning genes, which are involved in *Onthophagus* horn formation, suggesting the early redeployment of this subset of genes during the scarab horn evolution.

To understand how, molecularly, beetle horns have diversified, we are extending our analyses to include additional beetle species with different types of exaggerated horns, including rhinoceros beetles with diverse horn structures as well as horned beetles in other phylogenetic groups (Figure 3).

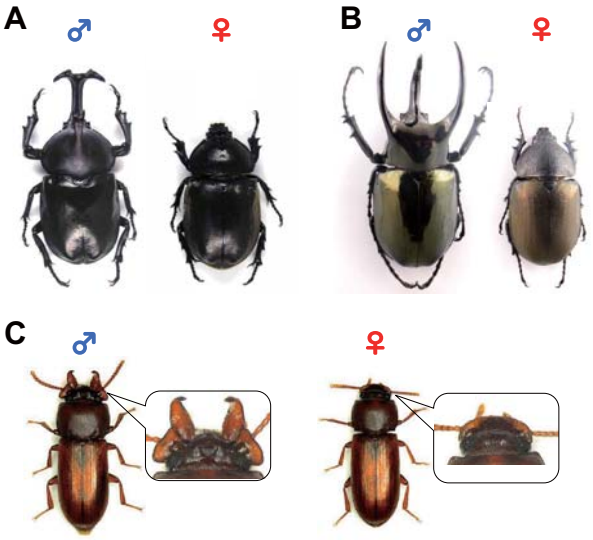

Figure 3. The Japanese rhinoceros beetle, *Trypoxylus dichotomus* (A), the Atlas beetle, *Chalcosoma atlas* (B) and the broad-horned flour beetle, *Gnatocerus cornutus* (C). Adult male (Left) and female (Right).

Publication List:

〔Original paper〕

● Matsuda, K., Gotoh, H., Tajika, Y., Sushida, T., Aonuma, H., Niimi, T., Akiyama, M., Inoue, Y., and Kondo, S. (2017). Complex furrows in a 2D epithelial sheet code the 3D structure of a beetle horn. Sci. Rep., *7*, 13939.

LABORATORY OF BIORESOURCES

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NARUSE, Kiyoshi

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Postdoctoral Fellow:	*YOKOI, Saori* *KAJIURA-KOBAYASHI, Hiroko*
Research Fellow:	*KANEKO, Hiroyo*
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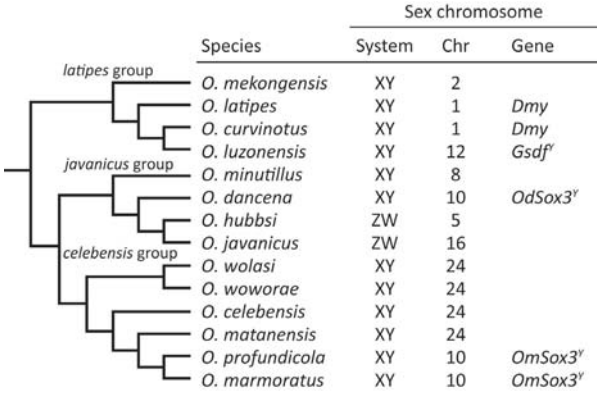
Secretary:	*SUZUKI, Tokiko*

Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on the evolution of the sex determination system using medaka and relatives, adaptive significance of mate-guarding behavior, The molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species and the identification of causal genes for pigment cell mutants. In addition to these activities, our laboratory has stepped forward to lead the National BioResource Project Medaka (NBRP Medaka) from 2007.

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

Sex chromosomes harbor a primary sex-determining signal that triggers sexual development of the organism. In mammals, *Sry* is the dominant male-determining gene located on the Y chromosome, and has evolved from the neural gene *Sox3* on the X chromosome probably through a regulatory mutation. However, independent evolution of sex chromosomes is widespread in non-mammalian vertebrates, suggesting that sex determination mechanisms are regulated by different genes and have evolved rapidly. Medaka fishes in the genus *Oryzias* have different sex chromosomes with different systems (XY and ZW), providing ideal conditions for investigating the mechanisms that lead to the rapid turnover of sex chromosomes (Figure 1). So far, different sex-determining genes, *Dmy* and *Gsdf*^{Y}, have been isolated from the *Oryzias* species, demonstrating that turnover of sex chromosomes is associated with the substitution of master sex-determining genes. Recently, we identified *Sox3* as a novel sex-determining gene on the XY sex chromosomes in the marine medaka *Oryzias dancena/melastgma* by positional cloning. Sex reversed phenotypes in transgenic fish and loss-of-function mutants of the Y chromosomal *Sox3* allele all point to its critical role in sex determination, suggesting that the neo-Y chromosome of *O. dancena* arose by co-option of *Sox3*. Furthermore, we found the *Sox3* gene also on the XY sex chromosomes in distantly related *Oryzias* species, *O. marmoratus* and *O. profundicola*. Fine mapping and association analysis identified the Y chromosome-specific 430-bp insertion at the *Sox3* locus, which appeared to be involved in its male determination function. The *Sox3*-dependent sex determination system in *Oryzias* species is polyphyletic, and the Y-specific insertion has not been found in *O. dancena*, suggesting that *Sox3* has evolved as the sex-determining gene independently in different lineages of *Oryzias*. These results suggest that *Sox3* might have acquired the novel male-determining function repeatedly and independently during vertebrate evolution.



	Species	Sex chromosome System	Chr	Gene
latipes group	*O. mekongensis*	XY	2	
	O. latipes	XY	1	*Dmy*
	O. curvinotus	XY	1	*Dmy*
	O. luzonensis	XY	12	*Gsdf*^{Y}
javanicus group	*O. minutillus*	XY	8	
	O. dancena	XY	10	*OdSox3*^{Y}
	O. hubbsi	ZW	5	
	O. javanicus	ZW	16	
celebensis group	*O. wolasi*	XY	24	
	O. woworae	XY	24	
	O. celebensis	XY	24	
	O. matanensis	XY	24	
	O. profundicola	XY	10	*OmSox3*^{Y}
	O. marmoratus	XY	10	*OmSox3*^{Y}

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

II. Identification of the causal gene of the medaka iridophore mutant, *guanineless(gu)*

The medaka has four pigment cell types: black melanophores, yellow xanthophores, white leucophores, and silvery iridophores. To date, causal genes of melanophore, xanthophore, and leucophore mutants have been elucidated, but the causal gene for the iridophore mutant remains unknown. Herein, we describe the iridophore mutant *gu*, which exhibits a strong reduction in visible iridophores throughout its larval to adult stages. The *gu* locus was previously mapped on chromosome 5, but was located near the telomeric region, making it difficult to integrate into the chromosome. We sought the causal gene of *gu* using synteny analysis with the zebrafish genome and found a strong candidate, *purine nucleoside phosphorylase 4a*. Gene targeting and a complementation test showed that *pnp4a* is the causal gene of *gu*. This result will allow the establishment of inbred medaka strains or other useful strains with see-through phenotypes, without disrupting the majority of the genetic background of each strain.

III. Adaptive significance of persistent mate-guarding behavior in medaka

Males of various animals exhibit mate-guarding behavior to prevent rival males from mating with the female. Most of them, however, exhibit this behavior during only the mating period, because persistent mate-guarding is thought to have a high energy cost, which would reduce male survival rate. Previously, we reported that medaka males exhibit mate-guarding irrespective of the mating period (Yokoi et al.,

2015) and it remains unknown whether there is some benefit of the persistent mate-guarding, such as enhancement of male reproductive success, in medaka fish. In addition, medaka females tend to choose visually-familiarized males as their mating partner and the adaptive significance of this female mating preference is totally unknown. Recently, we found that mate-guarding led to familiarization with the female while at the same time blocking the female's visual familiarization with other males in medaka fish. We put three fish (female, male, male) separately in a transparent three-chamber tank, which allowed the male in the center (near male) to maintain a closer proximity to the female than the other male (far male). Placement of the wild-type male in the center blocked visual familiarization of the far male by the female via mate-guarding. In contrast, placement of an arginine-vasotocin receptor mutant male, which exhibits mate-guarding deficits, in the center, allowing for maintaining a closer proximity to the female by the far male and did not block familiarization of the far male with the female. This finding suggested that persistent mate-guarding allows males to gain familiarity with the female over their rivals, which may enhance female preference for the dominant male (Figure 2). We hope that this study helps us understand the evolutional origin of "pair-bonding".

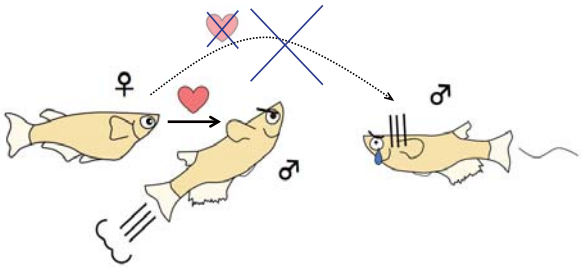


Figure 2. Persistent mate-guarding blocks familiarization between the female and the rival male, which may enhance female preference for the dominant male.

IV. The molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species endemic to Sulawesi, Indonesia

Sexual dimorphism is prevalent, but often differs remarkably between closely related species. However, we know little about which genetic changes can actually contribute to diversification of sexually dimorphic traits between closely related species. We therefore started to investigate the molecular genetic mechanisms underlying the diversification of sexually dimorphic body colorations in medaka (genus *Oryzias*) from Sulawesi, Indonesia. As a first step, we focused on an endemic species *Oryzias woworae*, whose males show red in the pectoral fins and blue in the lateral body. Quantitative trait loci (QTL) mapping in a F_2 intercross between a male of *O. woworae* and a female of *O. celebensis*, which is a close relative without such sexually dimorphic colorations, revealed that an autosomal locus controls red pigmentation in the pectoral fins. Subsequent gene expression analysis showed that upregulation of a gene on the QTL by the *cis*-regulatory mutation(s) would be responsible for the red fins. Further analysis with targeted genome editing technologies are underway to elucidate the molecular and developmental mechanisms underlying the red fin traits.

Publication List:

〔Original papers〕

- Ansai, S., Hosokawa, H., Maegawa, S., Naruse, K., Washio, Y., Sato, K., and Kinoshita, M. (2017). Deficiency of serotonin in raphe neurons and altered behavioral responses in *tryptophan hydroxylase 2*-knockout medaka (*Oryzias latipes*). Zebrafish *14*, 495-507.
- Inoue, Y., Saga, T., Aikawa, T., Kumagai, M., Shimada, A., Kawaguchi, Y., Naruse, K., Morishita, S., Koga, A., and Takeda, H. (2017). Complete fusion of a transposon and herpesvirus created the *Teratorn* mobile element in medaka fish. Nat. Commun. *8*, 551.
- Kimura, T., Takehana, Y., and Naruse, K. (2017). Pnp4a is the causal gene of the medaka iridophore mutant guanineless. G3-Genes Genomes Genet. *7*, 1357-1363.
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- Sasado, T., Kondoh, H., Furutani-Seiki, M., and Naruse, K. (2017). Mutation in *cpsf6/CFIm68* (*Cleavage and Polyadenylation Specificity Factor Subunit 6*) causes short 3'UTRs and disturbs gene expression in developing embryos, as revealed by an analysis of primordial germ cell migration using the medaka mutant *naruto*. PLoS ONE *12*, e0172467.
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〔Original paper (E-publication ahead of print)〕

- Nakamoto, M., Shibata, Y., Ohno, K.. Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. 2017 July 13.

〔Review article〕

- Okuyama, T., Yokoi, S., and Takeuchi, H. (2017). Molecular basis of social competence in medaka fish. Develop. Growth Differ. *59*, 211–218.

LABORATORY OF MORPHODIVERSITY

Associate Professor
KODAMA, Ryuji

Visiting Scientist: *YOSHIDA, Akihiro*

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

I. Wing outline shape formed by cell death

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

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

Cell death in the degeneration region proceeds very rapidly and finishes in a half-day to one-day period in *Pieris rapae* and in several other examined species. It was shown that the dying cells in the degeneration region have characteristics in common with the apoptotic cell death in mammalian cells, such as fragmented and condensed nuclei containing short DNA fragments detected by TUNEL staining. The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. The macrophages seem to be concentrated beneath the degeneration region by the strong adhesion between the basal surfaces of the dorsal and ventral epithelium in the differentiation region, which occurs at the time of prominent cell death and excludes macrophages from the differentiation region. Thus 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 (Yoshida and Emoto, Zool. Sci. *28*, 430-437, 2011).

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 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.

II. Wing morphogenesis and the growth of marginal scales in small moths

In the small moths which have very long scales along their wing margins, the cuticle of the pupal wings does not appear to be large enough to house these scales. We examined the developmental process of the pupal wings of three species of the small gelechiid moths and found that, concomitant with the programmed cell deaths at the wing margin, there occurs a shrinkage of the differentiation region which has not been observed in large winged butterflies and moths. The shrinkage concomitant with the cell deaths causes the space between the pupal wing and the cuticle to extend, which appears to contribute to the growth of long marginal scales.

Microscopic observation of the long marginal scales of *Phthorimaea operculella* revealed that they have a novel branching morphology and the branches were ubiquitously and densely distributed within the scale array to form a mesh-like architecture similar to a nonwoven fabric. The marginal scales maintain a coherent sheet-like structure during wingbeat (Figure 1).

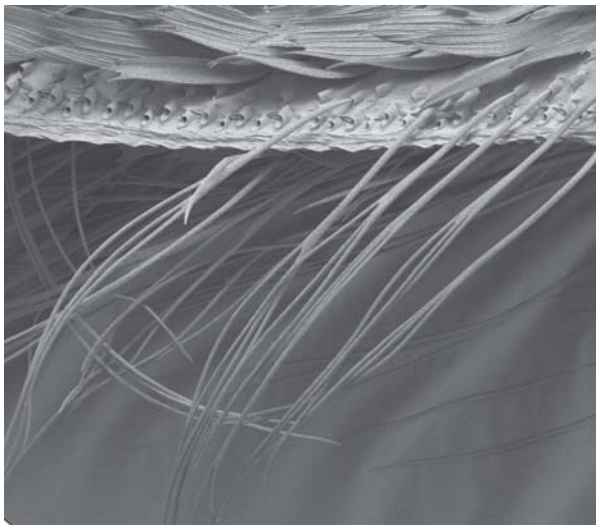

Figure 1. Scanning electron microscopy of the wing margin of *P. operculella*. Long marginal scales show branching morphology.

Publication List:

〔Original paper〕

● Yoshida, A., Tejima, S., Sakuma, M., Sakamaki, Y., and Kodama, R. (2017). Coherent array of branched filamentary scales along the wing margin of a small moth. Sci. Nat. *104*, 27

LABORATORY OF BIOLOGICAL DIVERSITY

MANO Group

Associate Professor: *MANO, Shoji*
Assistant Professor: *MANO, Shoji**
Postdoctoral Fellow: *KANAI, Masatake*
Visiting Scientist: *KAMIGAKI, Akane*
Technical Assistant: *HIKINO, Kazumi*
KATO, Kyoko
Secretary: *UEDA, Chizuru*

Plant cells can induce, degenerate and differentiate their organelles to adapt to environmental changes. This flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aims of our research group are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, and to understand the integrated functions of individual plants through organelle dynamics.

I. Molecular mechanisms of peroxisome dynamics and functions in plant cells

Peroxisomes are single-membrane bounded organelles, which are ubiquitously present in eukaryotic cells, and they are involved in various biological processes such as lipid metabolism and photorespiration. To understand peroxisome dynamics and functions, we have been analyzing a number of Arabidopsis mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants). Based on the analyses using these mutants a part of the mechanism of division, protein transport, degradation of peroxisomes, and the interactions of peroxisomes with other organelles were revealed (Figure 1). In addition, we found that peroxisomes are involved in the reproductive process. Therefore, peroxisome dynamics in gametes and gametophytes are currently under investigation.

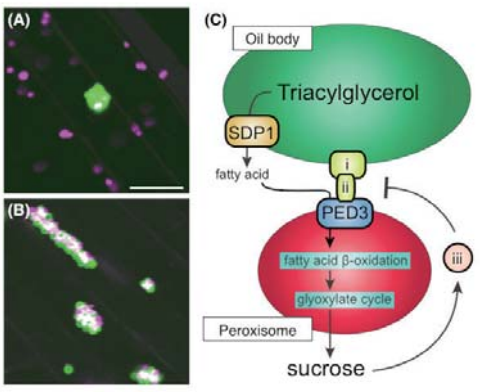

Figure 1. Physical interaction of peroxisomes and oil bodies. (A, B) Fluorescence observation of 3-day-old cotyledons of Arabidopsis wild-type (A) and *sdp1 (sucrose dependent 1)* plants (B). Magenta and green signals represent peroxisomes and oil bodies, respectively. Bar: 20 μm. (C) Model of the interaction of peroxisomes and oil bodies. PED3 (Peroxisome Defective 3) is the potential anchor protein on peroxisomal membranes. Sucrose acts as a negative signal for the interaction between both organelles.

II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves as foods and industrial materials. Storage reserves are different among different plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybean contains proteins as a major reserve. We are analyzing the mechanisms controlling oil and protein contents in seeds, and trying to apply our knowledge and techniques for increasing beneficial storage reserves.

III. Construction of The Plant Organelles Database 3 (PODB3)

PODB3 was built to promote a comprehensive understanding of organelle dynamics. PODB3 consists of six individual units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. Through these databases, users can obtain information on plant organelle responses to environmental stimuli of various tissues of several plant species, at different developmental stages. We expect that PODB3 will enhance the understanding of plant organelles among researchers.

Publication List:

〔Original papers〕

- Aboulela, M., Tanaka, Y., Nishimura, K., Mano, S., Kimura, T., and Nakagawa, T. (2017). A dual-site gateway cloning system for simultaneous cloning of two genes for plant transformation. Plasmid *92*, 1-11.
- Aboulela, M., Tanaka, Y., Nishimura, K., Mano, S., Nishimura, M., Ishiguro, S., Kimura, T., and Nakagawa, T. (2017). Development of an R4 dual-site (R4DS) gateway cloning system enabling the efficient simultaneous cloning of two desired sets of promoters and open reading frames in a binary vector for plant research. PLoS ONE *12*, e0177889.
- Hayashi, M., Tanaka, M. Yamamoto, S., Nakagawa, T., Kanai, M., Anegawa, A., Ohnishi, M., Mimura, T., and Nishimura, M. (2017). Plastidial folate prevents starch biosynthesis triggered by sugar influx into non-photosynthetic plastids of Arabidopsis. Plant Cell Physiol. *58*, 1328-1338.
- Kanai, M., Mano, S., and Nishimura, M. (2017). An efficient method for the isolation of highly purified RNA from seeds for use in quantitative transcriptome analysis. J. Vis. Exp. *119*, e55008.
- Watanabe, E., Mano, S., Yamada, K., Nishimura, M., Iuchi, S., Kobayashi, M., Uemura, M., and Kawamura, Y. (2017). Physiological analysis of Arabidopsis ecotype to investigate the freezing tolerance after cold acclimation process. Cryobiol. Cryotechnol. 63, 161-164.

〔Review article〕

- Watanabe, E., Mano, S., Hara-Nishimura, I., Nishimura, M., and Yamada, K. (2017). HSP90 stabilizes auxin receptor TIR1 and ensures plasticity of auxin responses. Plant Signal. Behav. *12*, e1311439.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Among various nutrients amino acids are the major nitrogen source; therefore, perception of the amino acid environment is essential for cells. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. TORC1 is involved in amino acid sensing, regulating protein synthesis, the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. So far, it is not clear whether TORC2 also perceives nutrient signals.

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

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

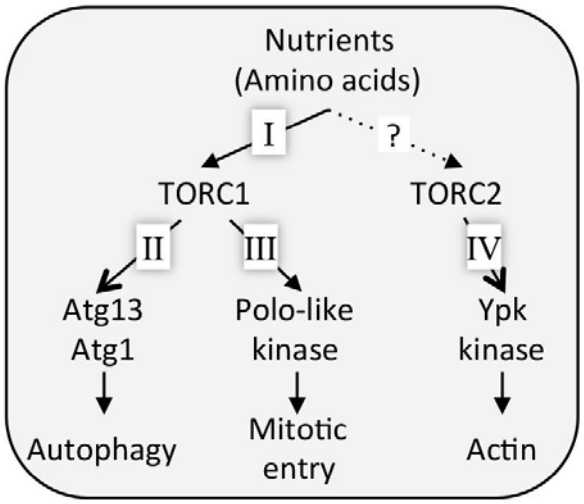

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

TORC1 is regulated by amino acids. Amino acids are fundamental nutrients, and 20 species of amino acids building proteins are not interchangeable with each other. Therefore, TORC1 should sense each amino acid individually. Mammalian mTORC1 is believed to be controlled by Rag GTPases and their regulators. However, Rag-factors are dispensable for amino acid sensing by TORC1 in the budding yeast, suggesting an alternative mechanism of TORC1 regulation.

Genetic investigation was done to discover the involvement of (aminoacyl-)tRNA in TORC1 regulation. Biochemical *in vitro* TORC1 assay also revealed that tRNA directly inhibits TORC1 kinase activity. Reducing cellular tRNA molecules desensitizes TORC1 inactivation by nitrogen starvation *in vivo*. Based on these results, a TORC1 regulatory model is proposed that free tRNA released from protein synthesis under amino acid starvation inhibits TORC1 activity. Therefore, TORC1 employs a tRNA-mediated mechanism to monitor intracellular amino acids.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

TORC1 negatively regulates autophagy, a protein degradation system induced by nutrient starvation.

We found the TORC1-mediated regulatory mechanism of autophagy. Under nutrient-rich conditions, TORC1 directly phosphorylates Atg13, a component of the Atg1 kinase complex. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced in response to TORC1 inactivation. Activation of Atg1 requires formation of the Atg1 complex. Phosphorylation of Atg13 by TORC1 plays a pivotal role in Atg1 complex formation; phosphorylated Atg13 loses its affinity to Atg1 resulting in disassembly of the Atg1 complex and repression of autophagy. On the other hand, dephosphorylation of Atg13 triggers formation of the Atg1 complex, activation of Atg1 kinase, and consequently induction of autophagy.

III. TORC1 regulates mitotic entry via polo-like kinase

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at the G1 phase.

We demonstrated that TORC1 is also involved in another stage of the cell cycle, mitotic entry. Cdc5, the yeast polo-kinase mediates this regulation, and the nuclear localization of Cdc5 at G2/M transition is controlled by TORC1. In addition, we discovered a physiological role of TORC1 in mitosis; autophagy negatively controlled by TORC1 plays an important part in maintenance of genome stability under starvation conditions.

IV. Ypk kinase acts directly downstream of TORC2 to control actin organization

TORC2 has an essential function controlling polarity of the actin cytoskeleton. We found that Ypk2, a member of the AGC kinase family is directly phosphorylated by TORC2. The activated allele of *YPK2* can rescue a lethality caused by TORC2 dysfunction, suggesting that Ypk kinase is the major downstream protein of the TORC2 pathway.

Publication List:

〔Original paper〕

● Kamada, Y. (2017). Novel tRNA function in amino acid sensing of yeast Tor complex1. Genes Cells 22,135-147.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

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

I. Gonadotropins in the starfish, *Patiria pectinifera*

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

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

II. Search for reproductive hormones in echinoderms

In a collaborative effort with Prof. Yoshikuni's Laboratory of Kyushu Univ., we are searching for reproductive hormones in echinoderms; starfishes, brittle stars, sea urchins, sea cucumbers, and crinoids. The collaborators have been able to purify physiological materials which induce egg maturation from nerve extracts and analyze them with a protein sequencer and a tandem mass spectrometer in the analytical center of our institute. One of them, named cubifrin, an IWMGY-amide peptide, in the sea cucumber *Aposticopus japonicus*, the others are in preparation for publications.

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. Especially relaxin like peptide precursor genes and insulin/IGF like peptide precursor genes were identified from many species. We are producing these neuropeptides by biological methods, e.g. bacterial systems and yeast systems, for providing to collaborators for biological assays.

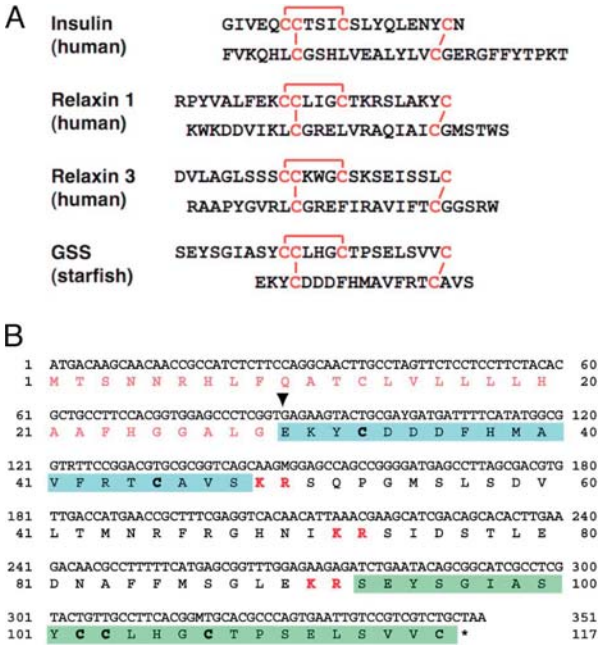

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

Publication List:

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

● Nakamoto, M., Shibata, Y., Ohno, K.. Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. 2017 July 13.

LABORATORY OF BIOLOGICAL DIVERSITY

KOMINE Group

Assistant Professor: *KOMINE, Yuriko*

We have been interested in the developmental and evolutional aspects of the structure of mammalian brains. In a comprehensive analysis of homeobox genes expressed in the developing mouse neocortex, we isolated a novel gene *Zfhx2*, which encodes a transcription factor containing three homeobox domains and 18 Zn-finger motifs. *Zfhx2* is highly expressed in the developing mouse brain, particularly in differentiating neurons, and continues to be expressed throughout adulthood at a low level. Two other phylogenically related genes, *Zfhx3* and *Zfhx4*, have been identified. The former was reported to be expressed in a manner dependent on neural differentiation, and the latter is a candidate gene causing congenital bilateral isolated ptosis. Although these three genes are expressed in substantially similar patterns in the developing brain, common functional features have not been clarified. Currently we have been focusing on *Zfhx2* to reveal its function and mechanisms of expression control in the developing brain.

I. Expression of *Zfhx2* is negatively regulated by its own antisense RNA

We found that the antisense strand of *Zfhx2* is also expressed in the mouse brain in a manner complementary to the expression of *Zfhx2* mRNA (Figure 1). Although most neurons express *Zfhx2* mRNA immediately after their final mitosis, several types of neuron (e.g., granule cells in the olfactory bulb and pyramidal and granule cells in the hippocampus) express antisense RNA prior to *Zfhx2* mRNA during the early phase of their differentiation. By generating a gene-targeting mouse line in which *Zfhx2* sense RNA is expressed but not antisense RNA, we showed that this antisense RNA has a negative regulatory role in the expression of *Zfhx2* mRNA. These observations suggest that the ZFHX2 protein might have a role in a particular step of neuronal differentiation, and in some types of neuron, this step might be delayed by the expression of antisense RNA.

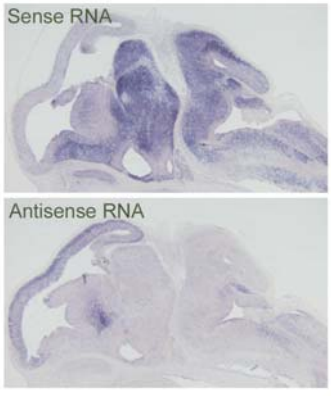

Figure 1. Expression of *Zfhx2* sense RNA (mRNA) and antisense RNA in the embryonic mouse brain. The antisense RNA was expressed where mRNA was not.

II. ZFHX2 might play roles in controlling emotional aspects

To elucidate the function of ZFHX2, we have also generated a *Zfhx2*-deficient mouse line. Although the production of the ZFHX2 protein is completely abolished in the homozygous mutant mice, the mice appear grossly normal and healthy. No anatomical abnormality has been observed in the mutant mouse brains so far examined. We hence subjected the *Zfhx2*-deficient mice to a comprehensive battery of behavioral tests to explore the physiological function of ZFHX2 in the nervous system. The homozygous *Zfhx2* deficient mice showed several behavioral abnormalities, namely, hyperactivity, enhanced depression-like behaviors, and an aberrantly altered anxiety-like phenotype. These behavioral phenotypes suggest that ZFHX2 might play roles in controlling emotional aspects through the function of monoaminergic neurons where ZFHX2 is expressed.

III. *ZFHX2* works also in pain perception process in human and mice

Recently, Cox's group in University College London found that a point mutation in the human *ZFHX2* gene segregates with the pain insensitivity found in a family with an inherited pain insensitive phenotype. Through the collaboration of several groups, including us, it has been shown that *ZFHX2* works as a critical gene for pain perception in humans and mice. Further work will resolve how the mutated *ZFHX2* gene contributes to the hypoalgesic phenotype and may help development of new analgesic drugs.

Publication List:

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

● Habib, A.M., Matsuyama, A., Okorokov, A.L., Santana, S., Bras, J.T., Aloisi, A.M., Emery, E.C., Bogdanov, Y., Follenfant, M., Gossage, S.J., Gras, M., Humphrey, J., Kolesnikov, A., Le Cann, K., Li, S., Minett, M., Pereira, V., Ponsolles, C., Sikandar, S., Torres, J.M., Yamaoka, K., Zhao, J., Komine, Y., Yamamori, T., Maniatis, N., Panov, K.I., Houlden, H., Ramirez, J.D., Bennett, D.L.H., Marsili, L., Bachiocco, V., Wood, J.N., and Cox, J.J. A novel human pain insensitivity disorder caused by a point mutation in *ZFHX2*. Brain 2017 Dec 14.

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

Assistant Professor: *HOSHINO, Atsushi*
Technical Assistant: *NAKAMURA, Ryoko*
TAKEUCHI, Tomoyo
ITO, Kazuyo

While genomic structures as well as their genetic information appear to stably transmit into daughter cells during cell division, and also into the next generation, they can actually vary genetically and/or epigenetically. Such variability has had a large impact on gene expression and evolution. To understand these genome dynamics in eukaryotes, especially in plants, we are characterizing the flower pigmentation of morning glories including *Ipomoea nil* (Japanese morning glory), *I. purpurea* (the common morning glory), and *I. tricolor*.

I. Flower pigmentation patterns

The wild type morning glories produce flowers with uniformly pigmented corolla, whereas a number of mutants displaying particular pigmentation patterns have been collected. Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate their flower pigmentation (Figure 1). We are currently characterizing detailed molecular mechanisms of these mutations.

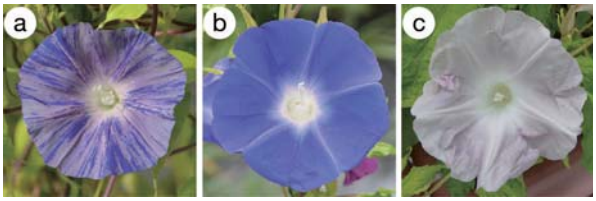

Figure 1. Flower phenotypes of the *duskish* mutant of *I. nil*. The phenotype is variable and displays variegated flowers (a), fully pigmented flowers (b), and pale grayish-purple flowers (c). As the flowers have the same genotype, epigenetic mechanisms are involved in the variable phenotypes.

II. Membrane transport of flower pigments

Anthocyanins are the most common flower pigments in Angiosperms including *I. nil*. They are synthesized in the cytosols and accumulate in the central vacuole in plant cells. Anthocyanin transport across the vacuolar membrane has long been debated. The transcriptional regulatory network of anthocyanin pigmentation supports involvement of an ATP binding caste (ABC) protein in the anthocyanin transport in *I. nil*. We started an international collaboration with researches in the Netherlands and Switzerland to reveal the function of the ABC protein.

III. Genome sequence information of the Japanese morning glory

In 2016, we reported an *I. nil* draft genome sequence with a scaffold N50 of 2.88 Mb, covering 98% of the 750 Mb genome. Scaffolds covering 91% of the genome sequence are anchored to 15 pseudo-chromosomes. A genome database for the genome sequence was built and opened to the public in 2017 (Figure 2). It includes a genome browser that enables users to analyze the 3,416 scaffolds (assembly name, Asagao_1.1), the 15 pseudo-chromosomes with the 3,095 scaffolds not anchored to the pseudo-chromosomes (Asagao_1.2), and the circular genomes of chloroplasts and mitochondria. The database provides BLAST, BLAT and keyword search services.

The genome sequence was also archived and used by several online databases and tools. The National Center for Biotechnology Information (NCBI) provides their own gene predictions (NCBI Ipomoea nil Annotation Release 100). The predicted genes are catalogued by the Kyoto Encyclopedia of Genes and Genomes (KEGG), and their functional information is visualized in the PATHWAY database that contains graphical representation of cellular processes (e.g. metabolism and signal transduction). Database Center for Life Science (DBCLS) equipped GGGenome and CRISPRdirect with the genome sequence. These tools facilitate ultrafast sequence search and rational design of CRISPR/Cas based genome editing target, respectively.

IV. BioResource of morning glories

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* is one of the most popular floricultural plants in Japan, and has a 100 year history of extensive genetic studies. Our collections include 240 lines and 160,000 DNA clones. The end sequences of the DNA clones can be viewed from the *I. nil* genome database (Figure 2).

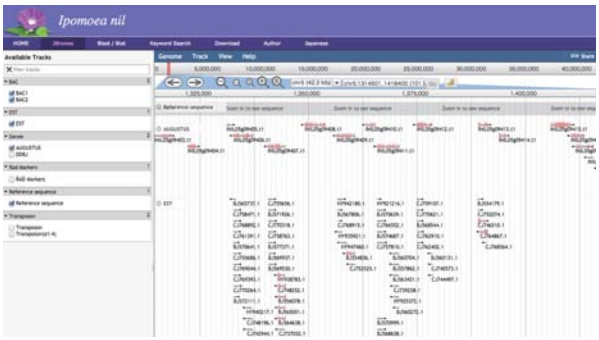

Figure 2. The *I. nil* genome database.
http://viewer.shigen.info/asagao/index.php

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*

Although transposons occupying a large portion of the genome in various plants were once thought to be junk DNA, they play an important role in genome reorganization and evolution. Active DNA transposons are important tools for gene functional analysis. The endogenous non-autonomous transposon, *nDart1-0*, in rice (*Oryza sativa* L.) is expected to generate various transposon-insertion mutants because *nDart1-0* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1-0* were promoted by an active autonomous element, *aDart1-27*, on chromosome 6. By using the endogenous *nDart1*/*aDart1-27* system in rice, a large-scale *nDart*-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation. The *nDart1*/*aDart1-27* system was introduced into a rice variety, Koshihikari, named MK-1. 3000 MK-1 plants were grown in field conditions (IPSR, Okayama Univ.). The genome of all plants were isolated for identifying insertion sites of *nDart1*.

I. Dominant mutation in rice

Seed size and number were controlled by various genes in the plants. It was reported that expression changes of high contribution genes for seed size, number and panicle shape resulted in decrease of total yield. A strategy for boosting rice yield based on molecular biology is to stack the finely tuned gene expressions. The *Lgg* mutant bore slightly large grains (Figure 1) as a dominant inheritance. Transposon-display identified the insertion site of the *Lgg* mutant.

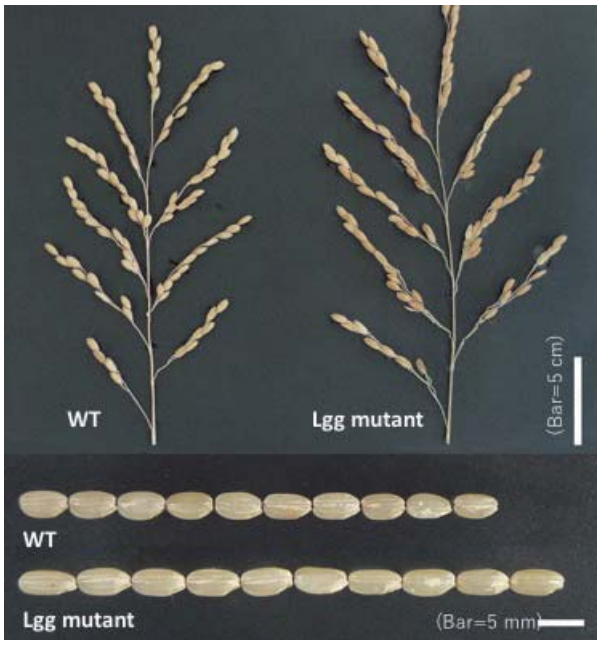

Figure 1. Phenotype of Large gain (Lgg). Harvested panicle and seeds.

II. Semidominant mutation in rice

The semidominant mutations produce the intermediate phenotype in individuals heterozygous for the gene concerned. The semidominant mutations were occasionally isolated from the MK-1, it was unclear what causes dominant mutations. Efficient selection and analysis of dominant mutants to analyze the gene functions in rice is very useful. Newly isolated, *Bushy dwarf tiller2* (*Bdt2*), which has the valuable agronomic traits of multiple tillering and dwarfism, was obtained from the MK-1 (Figure 2). Genetic analysis revealed the *Bdt2* mutation was controlled by two genetic elements. One *Bdt2* element, *Bdt2a* showed weak dwarf phenotype, another element, *Bdt2b* strengthened the effect of *Bdt2a*. A wild plant produces only 10 spikes or less, while *Bdt2a* produced 3 times as many, *Bdt2* produced 10 times the ears.

Figure 2. Phenotype of *Bushy dwarf tillers2* (*Bdt2*). (A) Two-month-old plants in the field (B) Segregants of *Bdt2* mutants. *Wild type* (left), *Bdt2a* (middle), *Bdt2*(right). (**C**) Abnormal bract of *Bdt2*.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

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

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

Our research interest is to understand the mechanism and regulation of chromosome condensation. We have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. Microscopic observation indicated the nucleolar localization of condensin. Consistent with this, the ribosomal RNA gene (rDNA) repeat is the most condensed region in the genome during mitosis. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. To date, the best characterized condensin binding region is the rDNA repeat on the right arm of chromosome XII in budding yeast. We further discovered the multiple protein network required to recruit condensin to the RFB site.

I. Dynamic relocalization of condensin during meiosis

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

II. Condensin-dependent chromatin folding

The RFB site, which consists of a ~150bp DNA sequence, is functioning as a cis-element for recruitment of condensin to chromatin in the yeast genome. If the RFB site is inserted into an ectopic chromosomal locus, condensin can associate with the ectopic RFB site. To explore the role of condensin in chromosome organization, we have constructed a strain in which two RFB sites are inserted on an ectopic chromosome arm with an interval of 15kb distance in the cell with complete deletion of chromosomal rDNA repeat. Using this strain, condensin-dependent chromatin interaction between two RFBs was examined by chromosome conformation capture (3C) assay. We found the condensin-dependent chromatin interaction between the two RFB sites on the chromosome arm. This result indicates that condensin plays a role in chromatin interaction between condensin binding sites, and this interaction leads to creation of a chromatin loop between those sites (Figure 1). It is thought that condensin-dependent chromatin folding is one of the basic molecular processes of chromosome condensation. During the cell cycle stages, the RFB - RFB interaction signal increases in metaphase and reaches its maximum level in anaphase. In addition to the RFB – RFB interaction, the chromatin interactions between internal regions of two RFBs increases in anaphase. Thus, the configuration of chromatin fiber changes from a simple loop into a complicated twisted shape as the cell cycle progresses from metaphase to anaphase.

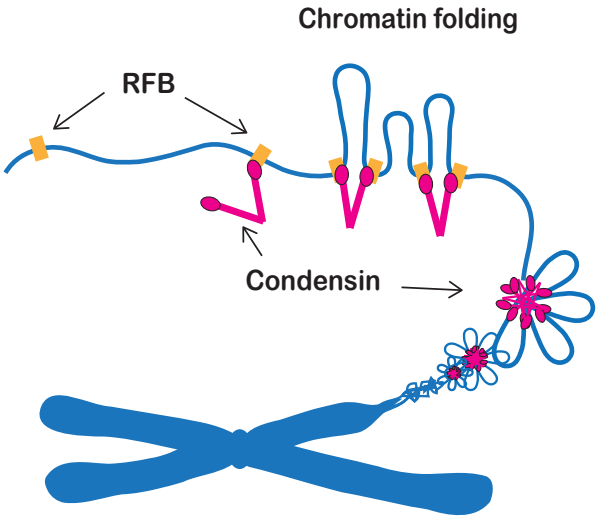

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

Specially Appointed Assistant Professor:
KATO, Kagayaki

Organogenesis is accomplished by a series of deformations of the planar cell sheet into a three-dimensional shape during embryogenesis. This drastic structural change is an integrated result of individual cell behaviors in response to spatio-temporally controlled mechanisms.

To better understand the programs underlying organ formation, it is required to analyze individual cells' morphology and dynamics quantitatively. However, due to the massive images generated by 4D microscopy and their ambiguity, this made it difficult to perform these analyses.

To unveil organogenesis from the point of view of distinct cell behaviors, we are developing application software that is capable of describing cell dynamics out of 4D time-lapse imaging data sets by employing image processing techniques.

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in the developing embryo is considered to be an essential model for collective cell migrations. Drastic cell rearrangements lead drastic structural changes to build elaborate organs such as the tubular network of *Drosophila* trachea. We are developing a software pipeline, which automatically recognizes individual cell shapes out of 3D space and tracks them through time. This system extracts cell boundaries and reconstitutes cell shapes as a skeletonized chain of voxels spanning 3D space. This abstract form of cell visualization makes it possible to describe morphometric quantities and kinetics of cells in single-cell resolution (Figure 1). These morphometric quantities allow us to perform comparative studies on shapes and behaviors precisely among several experimental conditions, to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system.

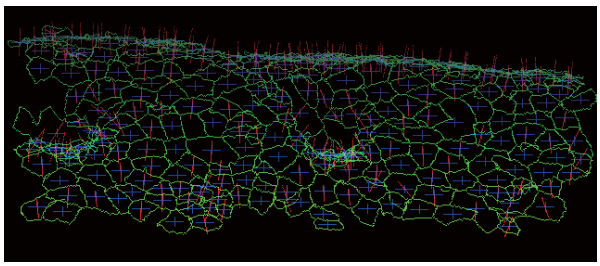

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta) are indicated for each cell.

II. Local image feature tracking for tissue deformation analysis

Besides cell boundary extraction, we also developed a derived algorithm for particle image velocimetry (PIV). This system is designed to measure tissue deformation even though the imaging constraints do not allow identification of individual cells out of images. This implementation detects structural characteristics, such as uneven fluorescence distributed over the specimen and tracks these patterns along a time-series. Despite that the tissue was labeled with non-targeted cytoplasmic GFP, this tracking software successfully outlined developmental dynamics of *Xenopus* neuroectoderm (Figure 2).

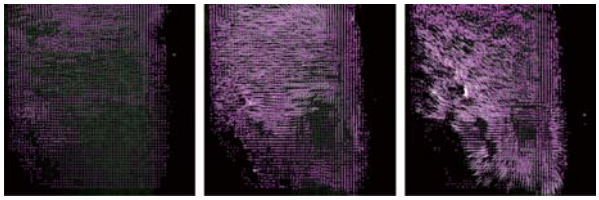

Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signals over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed the microscopy.

III. Software for manual image quantification

Biologically significant image features are not always significant to computational algorithms due to their structural instability. This kind of difficulty requires human eye inspection for feature extractions. A GUI (Graphical User Interface) application we developed can easily visualize 4D imaging data and has made manual feature annotations easy (Figure 3). This application is freely available at our website (https://is.cnsi.nins.jp/).

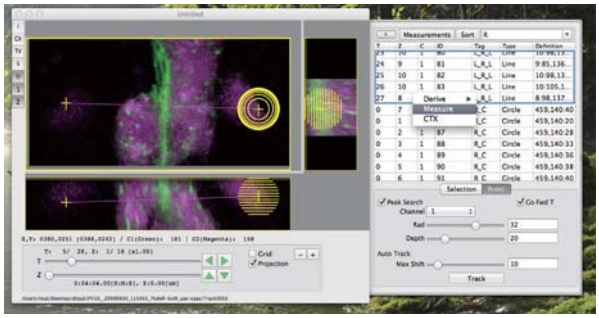

Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

LABORATORY OF BIOLOGICAL DIVERSITY

KIMORI Group

Specially Appointed Assistant Professor: *KIMORI, Yoshitaka*
Technical Assistant: *HYODO, Miwa*

Image processing methods significantly contribute to visualization of biomedical targets acquired from a variety of imaging techniques, including: wide-field optical and electron microscopy, X-ray computed tomography, magnetic resonance imaging and mammography. Quantitative interpretation of the deluge of complicated biomedical images, however, poses many research challenges. We have developed new computational methods based on mathematical morphology for quantitative image analysis. One of the most important purposes of image processing is to derive meaningful information, which is expressed as image structural properties. Mathematical morphology is a nonlinear image processing method based on set theory and is useful for the extraction of the structural properties from an image. It can be used as a fundamental tool to analyze biomedical images.

Novel contrast enhancement method based on mathematical morphology for medical diagnosis

Image processing is a crucial step in the analysis of medical imaging data. As such, it is fundamental to a wide range of biomedical imaging and clinical research fields. Image processing derives structural features, which are then numerically quantified by image analysis. Especially, in diagnostic imaging, contrast enhancement of structural details of lesion regions plays an important role. It improves image quality and supports clinician diagnosis. By using the contrast enhancement method, more accurate medical diagnosis can be expected. However, conventional image enhancement techniques also emphasize the noise and structure of various normal tissues other than lesion regions.

In this study, a contrast enhancement approach based on a new type of mathematical morphology is introduced. This method emphasizes only the structure of the lesion while suppressing the emphasis of noise and normal anatomical structure.

Mathematical morphology is a methodology for extracting shape and size information from an image. It involves configuration of a set of nonlinear operators that act on images by using structuring elements (SE). The SE, which indicates the shape characteristics in an image, is generally a small and simple binary image. The two basic morphological operators are dilation and erosion, from which many operations can be derived. However, since the size of lesions vary, in many cases it is not possible to process with fixed-size SE.

This proposed method is based on a morphological subtraction method. In this new type of morphological enhancement method, *h*-maxima transform is applied to the original medical image. The unwanted structures that surround the target are suppressed in the process of target enhancement. Furthermore, this new method has no restrictions on the size and shape of the target object that is to be enhanced.

This method was applied to enhancement of an abnormal region in the chest radiograph shown in Fig. 1. The arrow in Fig. 1(a) shows the position of the abnormality (lung nodule). Fig. 1(b) shows the image after contrast enhancement using the proposed method. From the result, the nodule region was clearly distinguished from the surrounding tissues.

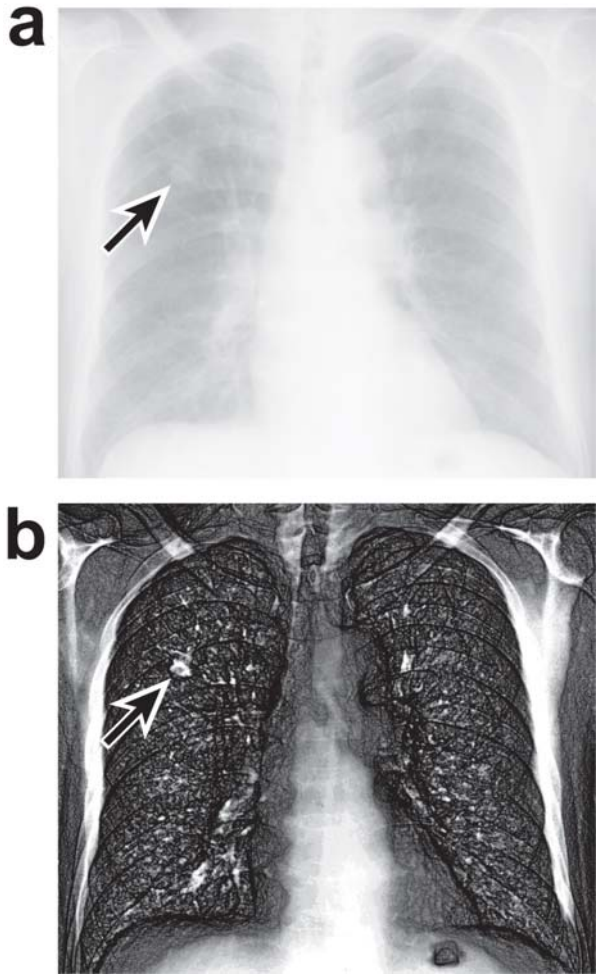

Figure 1. Contrast enhancement result for chest x-ray image: (a) Original chest radiograph. (b) Contrast enhanced image by using the proposed method. The image was obtained from a standard digital image database of chest lung nodules and non-nodules (Shiraishi et al., *ARJ Am. J. Roentgenol.* 174, 2000).

Publication List:

〔Original paper〕

● Osanai, Y., Shimizu, T., Mori, T., Yoshimura, Y., Hatanaka, N., Nambu, A., Kimori, Y., Koyama, S., Kobayashi, K., and Ikenaka, K. (2017). Rabies virus-mediated oligodendrocyte labeling reveals a single oligodendrocyte myelinates axons from distinct brain regions. Glia *65*, 93-105.

DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

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	KIDA, Emi
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	TOYAMA, Mami

Plants and algae have a large capacity to acclimate themselves to changing environments. We are interested in these acclimation processes, in particular, how efficiently yet safely they harness sunlight for photosynthesis under fluctuating light conditions. Using a model green alga, we are studying the molecular mechanisms underlying photoacclimation of the photosynthetic machinery. We are also applying the knowledge obtained in the studies of a model green alga to various phytoplankton, including symbiotic dinoflagellate, *Symbiodinium,* that associates with corals and sea anemones, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.

I. Non-photochemical quenching

Absorption of light in excess of the capacity for photosynthetic electron transport is damaging to photosynthetic organisms. Several mechanisms exist to avoid photodamage, which are collectively referred to as non-photochemical quenching (NPQ). This term comprises at least two major processes: state transitions (qT), the change in the relative antenna sizes of PSII and PSI, and energy-dependent quenching of excess energy (qE), the increased thermal dissipation triggered by lumen acidification. Recently, we isolated the PSII-LHCII supercomplex from both WT *C. reinhardtii* and the *npq4* mutant, which is qE-deficient and lacks the ancient light-harvesting protein LHCSR. LHCSR3 was present in the PSII-LHCII supercomplex from the high light-grown WT (Fig. 1) but not in the supercomplex from

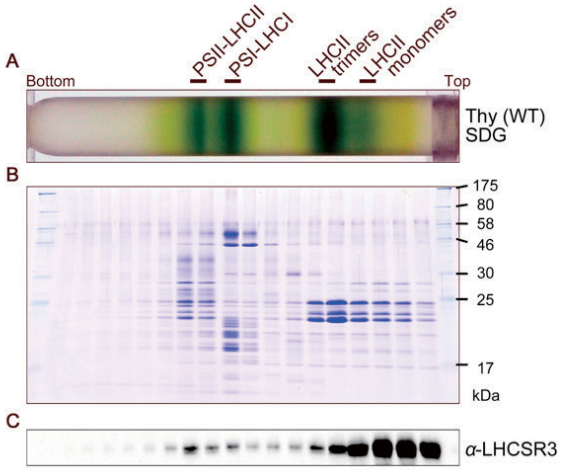

Figure 1. Purification of the PSII-LHCII-LHCSR3 supercomplex from WT C. *reinhardtii*. (A) Thylakoids from WT cells grown under high light conditions were subjected to sucrose density gradient centrifugation (SDG). (B) Polypeptides in the SDG fractions shown in (A) were analyzed by SDS/PAGE. (C) Polypeptides in the SDG fractions were subjected to immunoblotting with an antibody against LHCSR3.

the low light-grown WT or the *npq4* mutant. The purified PSII-LHCII supercomplex containing LHCSR3 showed a normal fluorescence lifetime at a neutral pH (7.5) by single-photon counting analysis but exhibited a significantly shorter lifetime (energy-quenching) at pH 5.5, which mimics the acidified lumen of the thylakoid membranes in high light-exposed chloroplasts. The switching from light-harvesting mode to energy-dissipating mode observed in the LHCSR3-containing PSII-LHCII supercomplex was inhibited by DCCD, a protein-modifying agent specific to protonatable amino acid residues. We conclude that the PSII-LHCII-LHCSR3 supercomplex formed in high light-grown *C. reinhardtii* cells is capable of energy dissipation upon protonation of LHCSR3. However, its molecular mechanism remains unclear.

To investigate how LHCSR3 dissipates excitation energy, thereby protecting the PSII supercomplex, we applied fluorescence decay–associated spectra (FDAS) analysis to a purified PSII–LHCII supercomplex with and without LHCSR3 from *Chlamydomonas reinhardtii*. We found that, when the PSII supercomplex is associated with LHCSR3 under high-light conditions, excitation energy transfer from light-harvesting complexes to chlorophyll binding protein CP43 is selectively inhibited compared with that to CP47, preventing excess excitation energy from overloading the reaction center. By analyzing femtosecond up-conversion fluorescence kinetics, we further found that pH- and LHCSR3-dependent quenching of the PSII—LHCII–LHCSR3 supercomplex is accompanied by a fluorescence emission centered at 684 nm, with a decay time constant of 18.6 ps, which is equivalent to the rise time constant of the lutein radical cation generated within a chlorophyll–lutein heterodimer. These results suggest a mechanism in which LHCSR3 transforms the PSII supercomplex into an energy-dissipative state and provide critical insight into the molecular events and characteristics in LHCSR3-dependent energy quenching (Fig. 2).

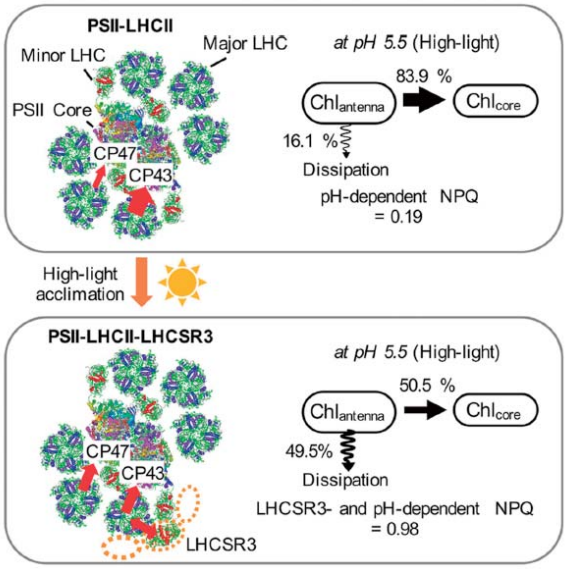

Figure 2. A proposed model of LHCSR3-dependent NPQ in PSII—LHCII–LHCSR3 supercomplexes of C. *reinhardtii*. LHCSR3 is expressed and associated with the PSII supercomplex under high-light conditions. The *circles of orange dots* represent the presumable binding site of LHCSR3. The binding of LHCSR3 to the PSII supercomplex inhibits the excitation energy transfer to CP43. At acidic pH, LHCSR3-dependent NPQ is activated, and it quenches excitation energy of LHCs. NPQ values of PSII–LHCII and PSII—LHCII–LHCSR3 were calculated from the difference between pH 7.5 and 5.5 conditions. Kim et al. (2017) *J. Biol. Chem. 17*, 18951-18960.

II. Species specificity in coral-algae symbiosis

Reef-building corals harbor endosymbiotic dinoflagellates of the genus *Symbiodinium* and rely on the energy that the algae generate from photosynthesis for their growth and survival. *Symbiodinium* are genetically diverse and their physiological characteristics (e.g., stress sensitivity) differ among phylotypes. Therefore, corals need to recruit *Symbiodinium* phylotypes that suit the environment in order to survive and adapt to changes (e.g., global change and warming). Interestingly, each coral species associate only with specific *Symbiodinium* phylotypes, consequently the diversity of symbionts available differs among coral species. However, the mechanism regulating the diversity of compatible symbionts in cnidarian organisms, including coral, was unknown.

We studied how corals select symbionts and what determines symbiont diversity in each coral species. In our study, we focus on the difference of cell size among *Symbiodinium* strains. Using a model *Aiptasia-Symbiodinium* system, we first found that, of *Symbiodinium* strains tested, only large-sized strains failed to infect the *Aiptasia* host. This size-dependency was supported by experiments using fluorescent microspheres of different sizes. We then tested the uptake of different sized *Symbiodinium* strains into aposymbiotic polyps from two different coral species. *Acropora tenuis* showed the same preference as *Aiptasia*, with no infection by the large-sized *Symbiodinium* strains. However, for *Cyphastrea serailia* all *Symbiodinium* strains tested, including the large-sized strains, were able to infect the host. Our results demonstrated that the infectivity of each *Symbiodinium* strains in a host is primarily determined by their cell size and that the diversity of symbionts in each host species is determined by their maximum acceptable symbiont cell size. We proposed that corals with a higher maximum threshold for symbiont cell size may have the opportunity to associate with more diverse *Symbiodinium* phylotypes. Such coral species may be better able to adapt to changing environmental conditions, and more specifically might be more suited to avoiding bleaching under increasing ocean temperatures.

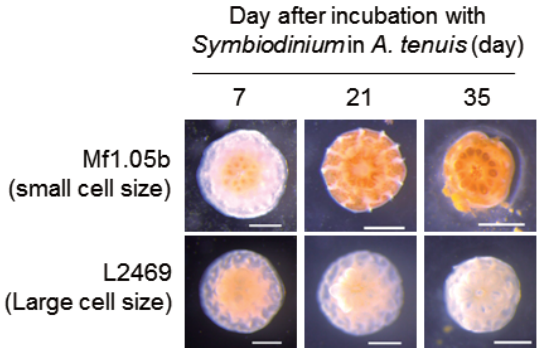

Figure 3. Infection of different Symbiodinium strains into corals. Aposymbiotic primal polyps of A. *tenuis* (scale bars, 500 μm) were separately incubated with small (Mf1.05b) and large (L2469) *Symbiodinium* strains. Uptake of *Symbiodinium* into coral polyps was monitored using a stereomicroscope. Biquand et al. (2017) *ISME J. 11*, 1702-1712.

Publication List:

〔Original Papers〕

- Biquand, E., Okubo, N., Aihara, Y., Rolland, V., Hayward, D., Hatta, M., Minagawa, J., Maruyama, T., and Takahashi, S. (2017). Acceptable symbiont cell size differs among cnidarian species and may limit symbiont diversity. ISME J. *11*, 1702-1712.
- Ho, S.-H., Nakanishi, A., Kato, Y., Yamasaki, H., Chang, J.-S., Misawa, N., Hirose, Y., Minagawa, J., Hasunuma, T., and Kondo, A. (2017). Dynamic metabolic profiling together with transcription analysis reveals salinity-induced starch-to-lipid biosynthesis in alga *Chlamydomonas* sp. JSC4. Sci. Rep. *7*, 45471.
- Kim, E., Akimoto, E., Tokutsu, R., Yokono, M., and Minagawa, J. (2017). Fluorescence lifetime analyses reveal how the high light-responsive protein LHCSR3 transforms PSII light-harvesting complexes into an energy-dissipative state. J. Biol. Chem. *292*, 18951-18960.
- Takizawa, K., Minagawa, J., Tamura, M., Kusakabe, N., and Narita, N. (2017). Red-edge position of habitable exoplanets around M-dwarfs. Sci. Rep. *7*, 7561.

DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

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	NAKATSUKASA, Mana
Visiting Undergraduate:	*MARUYAMA, Michiyo**
Visiting Scientist:	*GUH, Ying-Jey*
Technical Assistant:	*AKAMA, Akiko*
	KINOSHITA, Chie
	BABA, Nayumi
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Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize survival of their offspring in temperate zones. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. We use comparative approaches to understand these mechanisms. Medaka fish provide an excellent model to study these mechanisms because of their rapid and robust seasonal responses. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation.

I. Underlying mechanism that defines the critical photoperiod

It is well established that the circadian clock (i.e., an internal biological clock with a period of approximately 24 hrs) is somehow involved in seasonal time measurement.

However, it remains unknown how the circadian clock measures day length. It has been reported that Medaka populations that were caught at higher latitudes have more sophisticated responses to day length (Sawara and Egami, 1977). For example, Medaka fish caught in Hokkaido have a longer critical day length (i.e., duration of photoperiod required to cause a response) than those caught in Okinawa. To uncover the underlying mechanism of seasonal time measurement, we are currently performing a forward genetic analysis in Medaka populations collected from various latitudes all over Japan.

1-1 Variation in critical photoperiod with latitude in Medaka fish

To perform a forward genetic analysis, we have obtained 11 populations including wild populations, closed colonies, and inbred strains from all over Japan. We have examined the effects of changing day length to determine the critical day lengths that will cause seasonal responses in the gonad. In winter, fish were subjected to 10, 11, 12, 13, and 14 h day lengths with warm temperatures. Then gonadal development was examined to determine the critical day length.

As a result, we found differences in the critical day length among Medaka populations. That is, Medaka from higher latitudes required longer day lengths while those from lower latitudes required shorter day lengths.

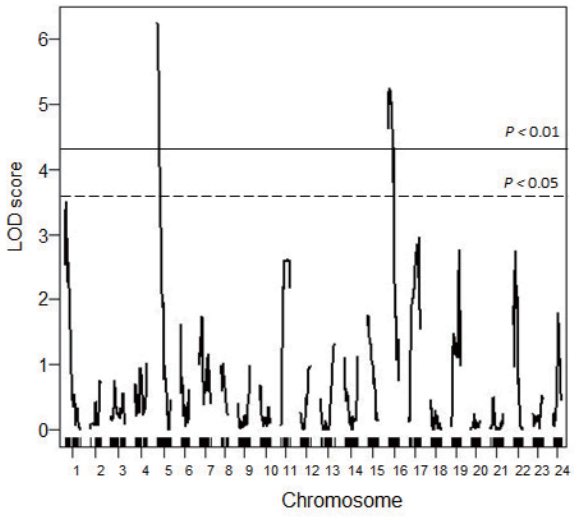

Figure 1. Result of QTL analysis for critical day length.

1-2 Quantitative trait loci (QTL) analysis of critical day length

To identify the genes regulating critical day length, quantitative trait loci (QTL) analysis was conducted using F_2 medaka derived from crosses between Northern and Southern populations. As a result, we identified significant QTLs using Restriction-site Associated DNA (RAD) markers (Figure 1). We have also performed whole genome re-sequencing using various Medaka strains that show different critical photoperiods, and identified potential candidate genes that define the critical day length.

II. Mechanism that determines seasonal breeders and non-seasonal breeders

Animals that reproduce year-round (e.g., human beings and laboratory mice) are so-called non-seasonal breeders. In contrast most animals living outside of tropical zones reproduce only during a particular period of the year. Therefore, they are called seasonal breeders. However, the underlying mechanism that determines seasonal breeders and non-seasonal breeders remains unknown. To uncover this mechanism, we performed a forward genetic approach.

2-1 Geographic variations in the responses to short day stimulus

When we transferred Medaka fish from summer conditions to winter conditions, we noticed that Medaka from lower latitudes do not regress their gonads even under short day conditions. Accordingly, we next examined the responses to short day conditions using 20 populations derived from various latitudes. As a result, populations from higher latitudes showed gonadal regression, while populations from lower latitudes did not regress their gonads (Figure 2).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

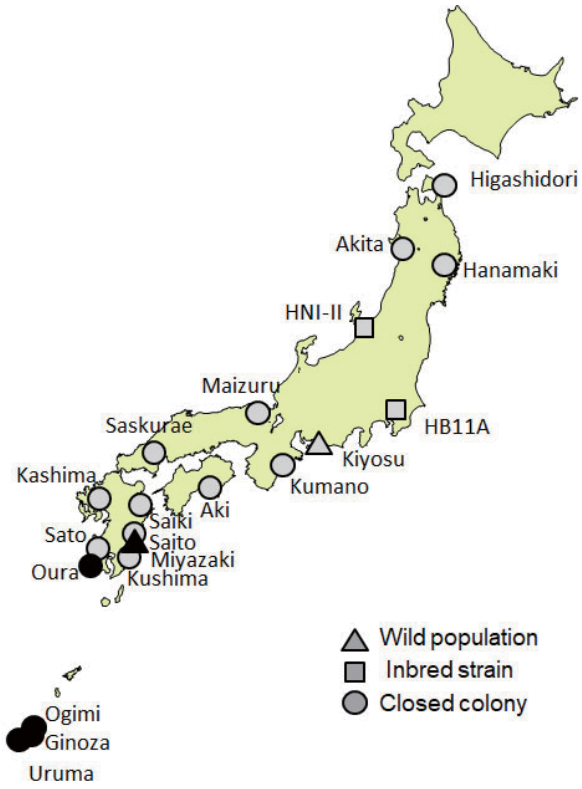

Figure 2. Medaka from lower latitudes (Solid black symbols) do not regress their gonads even under short day conditions.

2-2 QTL analysis of genes determining seasonal breeders and non-seasonal breeders

To identify genes that determine seasonal breeders and non-seasonal breeders, we performed QTL analysis using F_2 generations and identified a significant QTL that determines seasonal breeders and non-seasonal breeders. We are also performing a genome-wide association study to identify responsible genes.

III. Transcriptome analysis of seasonality in Medaka fish

In addition to the forward genetic approach, we have performed genome-wide transcriptome analysis of brain, eye, and liver of Medaka fish to understand the underlying mechanism of seasonal adaptation.

3-1. Seasonal changes in behaviors

Medaka kept under winter conditions stayed at the bottom of the tank, whereas medaka kept under summer conditions swam all over the tank. In general, fish avoid strong light stimulus (i.e., negative phototaxis). Summer medaka avoided white light stimulus, while winter medaka failed to show this negative phototaxis, suggesting that medaka are less sensitive to light under winter conditions, compared to summer conditions. When color preference was examined using three-dimensional computer graphics (3D-CG), summer medaka exhibited a preference for virtual fish with nuptial coloration, while winter medaka exhibited no such preference. This observation implies that the medaka's color perception is influenced by seasons.

3-2. Seasonal changes in phototransduction pathway

The temporal pattern of gene expression in medaka eyes associated with changes in seasons was examined by microarrays. This analysis identified summer induced genes that include various opsin genes and genes involved in downstream phototransduction pathways (Figure 3). We next examined the functional significance of these genes' expression using LWS (long wavelength sensitive) opsin-null fish. As a result, LWS opsin-null fish showed reduced negative phototaxis to white light compared to wild type fish under summer conditions, suggesting that summer-induced LWS opsin is critical for the emergence of negative phototaxis. In addition, LWS opsin-null fish showed weaker preference for virtual fish with nuptial coloration under summer conditions, suggesting that summer-induced LWS opsin is crucial for the emergence of mate preference observed in summer.

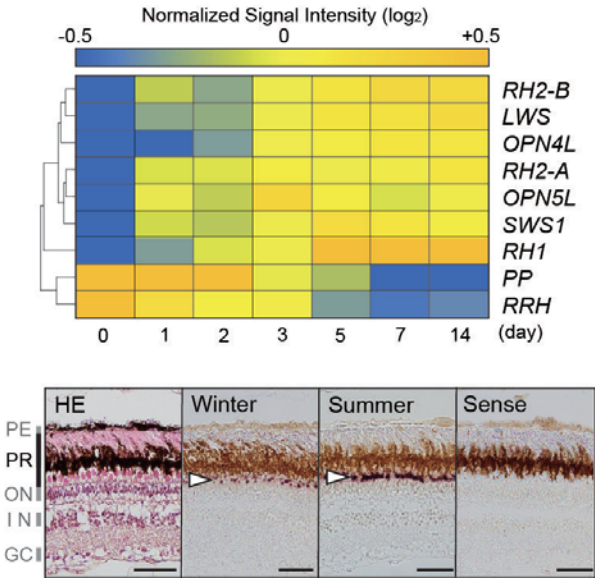

Figure 3. Genome-wide expression analysis reveals dynamic seasonal changes in opsin gene expression within the eye. Top: Results of microarray analysis. Bottom: Expression of LWS opsin within the retina.

Publication List:

〔Original paper〕

- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M, Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Commun. *8*, 412.

〔Review articles〕

- Ikegami, K., and Yoshimura, T. (2017). Molecular mechanism regulating seasonality. In: Biological Timekeeping: Clocks, Rhythms and Behaviour. 589-605.
- Tamai, K.T., and Yoshimura, T. (2017). Molecular and neuroendocrine mechanisms of avian seasonal reproduction. Adv. Exp. Med. Biol. *1001*, 125-136.

LABORATORY OF GENOME INFORMATICS

Assistant Professor
UCHIYAMA, Ikuo

Postdoctoral Fellow: *CHIBA, Hirokazu*

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

I. Microbial genome database for comparative analysis

The microbial genome database for comparative analysis (MBGD; URL http://mbgd.genome.ad.jp/) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes using the DomClust program combined with the DomRefine program (see Section III below). By means of these programs, MBGD not only provides comprehensive orthologous groups among the latest genomic data, but also allows users to create their own ortholog groups on the fly using a specified set of organisms. MBGD also has pre-calculated ortholog tables for each major taxonomic group, and provides several views to display the entire picture of each ortholog table. For some closely related taxa, MBGD provides the conserved synteny information calculated using the CoreAligner program. In addition, MBGD provides MyMBGD mode, which allows users to add their own genomes to MBGD. Moreover, MBGD now stores recently accumulating draft genome data, and allows users to incorporate them into a user specific ortholog database through the MyMBGD functionality

II. Hierarchical strategy for creating ortholog tables

Previously, MBGD provided two types of ortholog tables: the standard ortholog table containing one representative genome from each genus covering the entire taxonomic range, and the taxon specific ortholog tables containing the genomes belonging to each taxonomic group (species, genus, family and so on). Although, by this approach, most of the "core genes", i.e. genes well conserved within species or genus, can be incorporated into the standard ortholog table, the genes that are not conserved in the representative genomes cannot be incorporated. Considering the great diversity of microbial species/genus genomes, a considerable amount of information may be lost in the current standard ortholog table. To address this problem, we developed a stepwise protocol to construct orthologous relationships. In this approach, we first create a within-species ortholog table for each species and construct a species level pan-genome by picking one representative gene from each orthologous group (Figure. 1). Next, we create a within-genus ortholog table for each genus using as input the species-level pan-genomes generated in the previous step and construct a genus level pan-genome. Finally, we create an ortholog table covering the entire taxonomic range by comparison of the genus level pan-genomes. We used a rapid similarity search program, UBLAST, to calculate all-against-all similarities for within-species and within-genus comparisons, which can drastically reduce the computation cost. By this approach, we can integrate various pan-genomes into a single ortholog table, which enables us to analyze evolutionary processes that generate within-species or within-genus diversity of microbes.

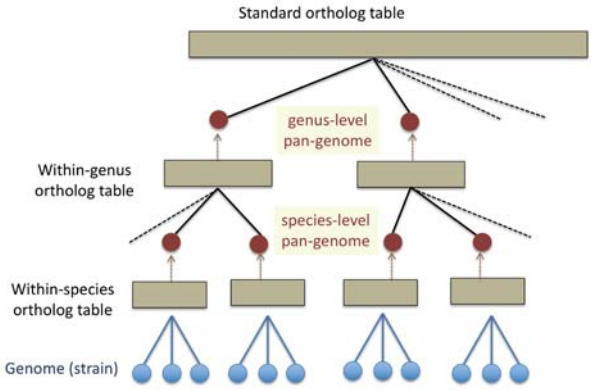

Figure 1. Hierarchical protocol for creating the standard ortholog table

III. Orthologous gene classification among multiple genomes at the domain level

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

We also developed a procedure to refine the DomClust classification based on multiple sequence alignments instead of pairwise sequence alignments. The method is based on a scoring scheme, the domain-specific sum-of-pairs (DSP) score, which evaluates domain-level classification using the sum total of domain-level alignment scores. We developed

a refinement pipeline to improve domain-level clustering, DomRefine, by optimizing the DSP score. DomRefine is now used to construct the standard ortholog table covering all the representative genomes stored in MBGD.

Domain-level classification is a unique feature of our ortholog classification system. In fact, it is different from conventional domain databases like Pfam in that it is based on orthology instead of homology. Particularly, this data is considered to be suitable for analyzing domain fusion events that occurred during evolution. Now, by analyzing the domain-level ortholog grouping data combined with taxonomic and functional information, we are trying to elucidate when and in what kind of genes domain fusion events frequently occurred.

IV. Development of a workbench for comparative genomics

We have developed a comparative genomics workbench named RECOG (Research Environment for COmparative Genomics), which aims to extend the current MBGD system by incorporating more advanced analysis functionalities. The central function of RECOG is to display and manipulate a large-scale ortholog table. The ortholog table viewer is a spreadsheet like viewer that can display the entire ortholog table, containing more than a thousand genomes. In RECOG, several basic operations on the ortholog table are defined, which are classified into categories such as filtering, sorting, and coloring, and various comparative analyses can be done by combining these basic operations. In addition, RECOG allows the user to input arbitrary gene properties and compare these properties among orthologs in various genomes.

We continue to develop the system and apply it to various genome comparison studies under collaborative research projects (including *H. pylori* genome comparison described in Section VI below). In particular, in addition to microbial genome comparison, we are trying to apply RECOG to comparative analyses of transcriptomic data and metagenomic data.

V. Ortholog data representation using the Semantic Web technology to integrate various microbial databases

Orthology is a key to integrate knowledge about various organisms through comparative analysis. We have constructed an ortholog database using Semantic Web technology, aiming at the integration of numerous genomic data and various types of biological information. To formalize the structure of the ortholog information in the Semantic Web, we developed an ortholog ontology (OrthO) and described the ortholog information in MBGD in the form of the Resource Description Framework (RDF). To further standardize the ontology, we developed the Orthology Ontology (ORTH) in collaboration with Dr. Fernandez-Breis (Univ. Murcia) by integrating OrthO and OGO (another ortholog ontology developed by Dr. Fernandez-Breis) and reusing other existing ontologies.

On the basis of this framework, we have integrated various kinds of microbial data using the ortholog information as a hub, as part of the MicrobeDB.jp project (http://microbedb.jp/) under the National Bioscience Database Center.

In addition, to facilitate the utilization of the RDF databases distributed worldwide, we developed a command-line tool, named SPANG. SPANG simplifies querying distributed RDF stores using the SPARQL query language, and provides a framework for reusing and sharing queries across the Web, thereby reducing the burden of writing complex queries in SPARQL.

VI. A novel approach for identification of genomic islands

Genomes of bacterial species can show great variation in their gene content, and thus systematic analysis of the entire gene repertoire, termed the “pan-genome”, is important for understanding bacterial intra-species diversity. We analyzed the pan-genome identified among 30 strains of the human gastric pathogen *Helicobacter pylori* isolated from various phylogeographical groups. We developed a method (FindMobile) to define mobility of genes against the reference coordinate determined by the core alignment created by CoreAligner, and classified each non-core gene into mobility classes. In addition, by clustering the accessory OGs on the basis of phylogenetic pattern similarity and chromosomal proximity, we identified 60 co-occurring gene clusters (CGCs). We are now trying to generalize this approach to identify genomic islands in various bacterial species.

Publication List:

〔Original papers〕

- Chiba, H., and Uchiyama, I. (2017). SPANG: A SPARQL client supporting generation and reuse of queries for distributed RDF databases. BMC Bioinformatics *18*, 93.
- Hayatsu, M., Tago, K., Uchiyama, I., Toyota, A., Wang, Y., Shimomura, Y., Okubo, T., Kurisu, F., Hirono, Y., Nonaka, K., Akiyama, H., and Takami, H. (2017). An acid-tolerant ammonia-oxidizing γ-proteobacterium from soil. ISME J. *11*, 1130-1141.
- Ikeda, T., Uchiyama, I., Iwasaki, M., Sasaki, T., Nakagawa, M., Okita, K., and Masui, S. (2017). Artificial acceleration of mammalian cell reprogramming by bacterial proteins. Genes Cells *22*, 918-928.
- Takami, H., Toyoda, A., Uchiyama, I., Itoh, T., Takaki, Y., Arai, W., Nishi, S., Kawai, M., and Ikeda, H. (2017). Complete genome sequence and expression profile of the commercial lytic enzyme producer *Lysobacter enzymogenes* M497-1, DNA Res. *24*, 169-177.

〔Review Article〕

- Uchiyama, I. (2017). Ortholog identification and comparative analysis of microbial genomes using MBGD and RECOG. In Protein function prediction, D. Kihara ed., Humana press, pp. 147-168.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

Postdoctoral Fellow: *TANIGUCHI, Atsushi*
Technical Assistant: *ISHIBASHI, Tomoko*

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

I. Initial step for left-right asymmetry

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

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

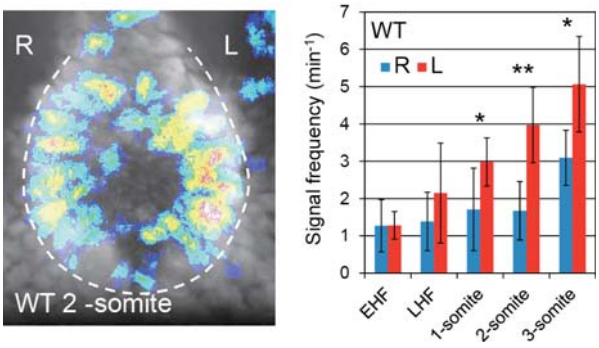

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

Based on these findings, we are now examining the validity of the two major proposed models of flow sensing, mechanosensing and chemosensing, and a third novel mechanism.

II. Development of light-sheet microscopy

Light-sheet microscopy has become popular over this decade for its advantages including low photodamage and fast image acquisition. We are now running two light-sheet microscopes, one commercial and the other self-made, maintaining them for both collaborations and for our own research interest, left-right asymmetry.

Over several years we have developed a fast light-sheet microscope named ezDSLM, in which the excitation light sheet and detection objective move instead of the specimen for z-scanning. We installed an electrically tunable lens (ETL) to achieve further speed and exclusion of mechanical vibrations that disturb continuous 4D imaging of floating or loosely held specimens.

Our light-sheet microscopes are open to other researchers via NIBB's Collaborative Research and MEXT's Advanced Bioimaging Support (ABiS) programs. Numerous collaborations are in progress including observation of moving *Amoeba proteus*, neuronal activity in *Drosophila* larvae, cell migration in zebrafish embryos, cleared mouse brains, and marine crustaceans, etc.

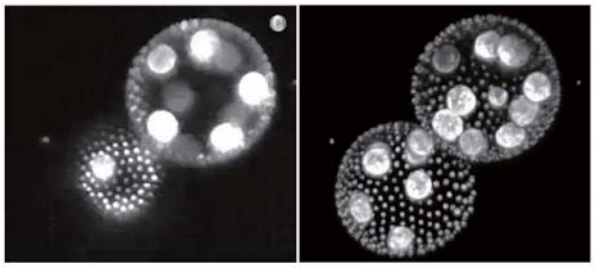

Figure 2. Images of floating volvox taken by ezDSLM with ETL. Left: Single optical section. Right: Maximum intensity projection.

Publication List:

〔Original papers〕

- Hashiura, T., Kimura, E., Fujisawa, S., Oikawa, S., Nonaka, S., Kurosaka, D., and Hitomi, J. (2017). Live imaging of primary ocular vasculature formation in zebrafish. PLoS ONE *12*, e0176456.
- Taniguchi, A., Kimura, Y., Mori, I., Nonaka, S., and Higashijima, S. (2017). Axially-confined in vivo single-cell labeling by primed conversion using blue and red lasers with conventional confocal microscopes. Dev. Growth Differ. *59*, 741-748.

LABORATORY OF NUCLEAR DYNAMICS

Specially Appointed Associate Professor
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Postdoctoral Fellow: *KURIHARA, Misuzu*
SOKENDAI Graduate Student: *ISHII, Satoko*
Visiting Graduate Student: *KAKIZUKA, Taishi*
Technical Assistant: *SANBO, Chiaki*
TAGAWA, Ayako
Secretary: *HACHISUKA, Midori*

A fundamental question in biology is to understand the mechanisms underlying cell-fate decision. Genomic reprogramming after mammalian fertilization reverts terminally differentiated gametes into toti- or pluri-potent states to start a new developmental program. Cell lineage allocation in the reprogramming process is accompanied by drastic changes in the pattern of gene expression, epigenetic configurations, and nuclear organization. We aim to reveal the roles of chromatin dynamics in cell lineage-allocation by deciphering the molecular mechanisms underlying remodeling of nuclear organization and their effects on developmental gene expression, using mouse embryos and embryonic stem (ES) cells as model systems.

Epigenetic reprogramming in early mouse embryos.

Genomic reprogramming reverts fully differentiated cells to a totipotent state to start a new developmental program. In the early mouse embryo, terminally differentiated gametes are reprogrammed after fertilization thereby acquiring a totipotent state. Upon the fourth cleavage, 8-cell stage embryos, which have undergone the process of compaction, will give rise to the morula (Figure 1). The outer cells of the morula will differentiate into the epithelial trophectoderm (TE) of the blastocyst. The inner cells of the morula will become the inner cell mass (ICM) of the blastocyst. The ICM subsequently leads to the formation of two lineages, epiblast (EPI) and primitive endoderm (PE), with the former representing pluripotency as it gives rise to the embryo itself. The reprogramming event is accompanied by epigenetic modifications and changes in chromatin structures throughout the embryo, which are essential for regulation of gene expression involved in differentiation, and reprogramming of the EPI.

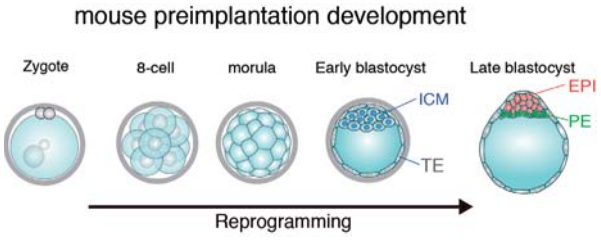

Figure 1. Lineage allocation in mouse preimplantation development

Remodeling of nuclear architecture in development

Chromatin is organized in a non-random fashion within three-dimensional nuclear space. During developmental processes, nuclear architecture is dramatically reconstructed, resulting in establishment of cell-type specific nuclear organization. Defects in structural components of the nucleus are responsible for developmental aberrations and several human diseases. Remodeling of nuclear architecture leads to spatial arrangement of genes, which could affect genome functions including gene expression. However, regulatory mechanisms underlying nuclear reorganization during cell-fate decision remains largely unknown.

Chromatin structure

Spatiotemporal organization of genomic DNA within the nucleus is suggested as an emerging key player to regulate gene expression. The developmental program accompanies nuclear remodeling, resulting in construction of cell-type specific nuclear architecture. Firstly, chromosomes are confined in discrete nuclear spaces, "chromosome territories" (Figure 2). Within them, further levels of 3D organization, "topologically associating domains" (TADs), are observed. TADs can be defined as linear units of chromatin containing several gene loci, and fold as discrete 3D structures in which gene loci frequently interact with each other. Recent works have revealed that folding of "local" chromatin structures such as enhancer-promoter looping is associated with genome functions. Despite the drastic changes of these hierarchical chromatin structures, their role in cell-fate decision remains largely unexplored.

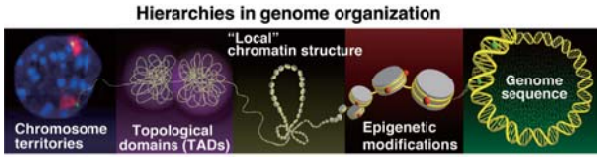

Figure 2. Hierarchical chromatin structure

Approach

We have developed a powerful imaging technology termed TALE-mediated Genome Visualization (TGV), which allows us to track specific genomic sequences in living cells (Miyanari Y, Nature Structural & Molecular Biology, 2013). Importantly, this technique is versatile and can be extended to allow many robust applications, which will be integrated into our study to manipulate several genome functions. Based on new technological development, we aim to understand biological roles of chromatin dynamics in cell-fate decision.

LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY

Specially Appointed Associate Professor
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Adjunct Professor (BIO-NEXT Project, OIIB): *TSUKAYA, Hirokazu*
Postdoctoral Fellow: *NOZAKI, Mamoru*
Visiting Graduate Student: *TOMOI, Takumi*
Technical Assistant: *YAMAGUCHI, Chinami*
Secretary: *HACHISUKA, Midori*

Development and metabolism are intertwined with one another during organogenesis. This interaction is essential to maintain the metabolic state in a developmental context-dependent manner. There is also a growing awareness that metabolism plays instructive roles in developmental processes. The emerging picture depicts metabolism as a critical system not only for sustaining physiological conditions, but also regulating developmental patterning by coordinating various cellular processes. However, it remains largely unclear how this interaction is achieved in multicellular organisms. We aim to reveal as-yet-unknown relationships between developmental and metabolic processes and their biological meaning by elucidating molecular mechanisms of the system. To address this, we use trans-omics approach including metabolome and transcriptome analyses using *Arabidopsis thaliana* as a model, in conjunction with standard molecular genetics and biochemistry techniques.

I. Cytochrome P450 epoxidase for embryonic patterning

To uncover hidden relationships between development and metabolism, we performed quantitative phenome screening using *A. thaliana* mutants of orphan cytochrome P450 genes. We identified the *cyp77a4* mutant, which exhibits irregular embryonic patterning as evidenced by defects, such as a cup-shaped cotyledon morphology (Figure 1). Although CYP77A4 is the first cytochrome P450 reported to be able to catalyze the epoxidation of fatty acids in plants, its function in development is completely unknown.

Through the use of auxin-related reporters, we determined that CYP77A4 is essential for polar auxin transport via proper localization of PIN1 (an auxin efflux carrier). Interestingly, unlike other enzyme mutants defective in auxin dynamics in ubiquitous tissues, the *cyp77a4* mutant was associated with defects specifically in embryos. Furthermore, our double mutant analysis clarified that *CYP77A4* and *CYP77A6* (the phylogenetically closest gene to *CYP77A4*) are functionally independent. Based on these findings, we proposed that the metabolic requirement for polarity establishment via auxin dynamics differ between tissues, and that in embryos this depends on a CYP77A4-dependent metabolic pathway. These findings may augment our understanding of fatty-acid epoxidation by uncovering a novel developmental function of the epoxidase (Kawade et al., *submitted*).

Figure 1. Irregular arrangement of cotyledons in the *cyp77a4* mutants. (A-E) WT-like (A), abnormally arranged (B), single (C and D), and cup-shaped (E) cotyledons in the *cyp77a4-3* mutants. Bars = 2 mm.

II. Developmental signal intertwined with metabolism

ANGUSTIFOLIA3 (AN3) is a transcriptional co-activator, which promotes cell proliferation in leaves. We recently showed that AN3 forms an expression gradient along a proximal-to-distal developmental axis to regulate cell proliferation dynamics in time and space (Kawade *et al*., 2017; Figure 2). To gain further insights into how AN3 contributes to tissue patterning, we conducted transcriptome and metabolome analyses using the *an3* mutants. We found that AN3 regulates a transcriptional network for oxygen homeostasis. Our metabolic profiling indeed detected characteristic features of redox disturbance in the *an3* mutants. Although cell proliferation is an essential process for tissue patterning, reactive oxygen species are generated through energy production. It would be interesting to assume that the AN3 signal may solve this dilemma, cell proliferation *vs*. oxidative stress, in plant development.

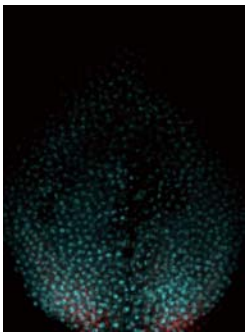

Figure 2. A merged image of the leaf primordia (around 200-μm length) expressing genetically engineered mobile or immobile AN3 (cyan or red, respectively). The distribution of the mobile AN3 gradually spreads along the leaf proximal-to-distal axis (from down to top), which is broader than that of the immobile one.

Publication List:

〔Original papers〕

- Kawade, K., Tanimoto, H., Horiguchi, G., and Tsukaya, H. (2017). Spatially different tissue-scale diffusivity shapes ANGUSTIFOLIA3 gradient in growing leaves. Biophys. J. *113*, 1109-1120.
- Kawade, K., and Tsukaya, H. (2017). Probing the stochastic property of endoreduplication in cell size determination of *Arabidopsis thaliana* leaf epidermal tissue. PLoS ONE *12*, e0185050.
- Sawada, Y., Tsukaya, H., Li, Y., Sato, M., Kawade, K., and Hirai, M.Y. (2017). A novel method for single-grain-based metabolic profiling of Arabidopsis seed. Metabolomics *13*, 75.

ASTROBIOLOGY CENTER

Specially Appointed Associate Professor
TAKIZAWA, Kenji

M-dwarfs, relatively small and cool stars, are the most abundant neighboring stars and thus could be the first candidates for searching habitable exoplanets and detecting a sign of extraterrestrial life. One of the most important biosignatures on an exoplanet is a specific reflection pattern on the land surface named 'red-edge' that is caused by land vegetation. On the Earth, red-edge appears between visible light, which is absorbed by photosynthetic pigments, and near infrared (NIR) radiation, which is reflected via leaf structure. In previous reports, it was predicted that red-edge could be red-shifted around M-dwarfs since phototrophs on the exoplanet should use abundant NIR radiation for photosynthesis in addition to visible light. We have proposed an alternative prediction that red-edge could be observed as on the Earth even on exoplanets if the initial oxygenic photosynthesis evolved underwater.

I. Photosynthetically active radiation on the exoplanets around M-dwarfs

We estimated the light conditions expected on habitable planets around an M-dwarf. Assuming that an Earth-like planet is located in the habitable zone around AD Leo, and M4.5eV star located 16 light years away from our solar system, the light conditions on the planet were estimated and compared with solar irradiation on the Earth. The land surface of the M-dwarf planet is illuminated by strong NIR-radiation, while Earth's surface is illuminated by visible light. On the other hand, similar light conditions are expected underwater since only blue-green light can penetrate meters of water (Figure 1).

The visible-light intensity obtained on the land surface of a hypothetical habitable planet around AD Leo is equivalent to that from Earth's ocean at a depth of 15 m. The lower limit of light intensity needed to support the growth of oxygenic marine phototrophs on Earth is about 20 nmol photon m^{-2} s^{-1}, which corresponds to a water depth of 166 m in the model calculation. The same amount of visible light is obtained at a water depth of 123 m on the AD Leo planet. If the origin of life and its early evolution is placed underwater, Earth-type oxygenic photosynthesis may evolve on exoplanets around M-dwarfs.

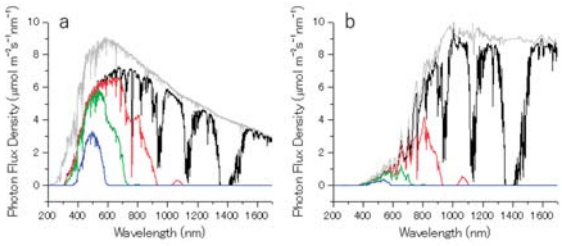

Figure 1. Photon flux density spectra for Earth (a) and a hypothetical habitable planet around AD Leo (b). Gray lines show the spectra obtained at the top of the atmosphere. Black lines show the spectra at the land surface passing through a 1.5 air-mass atmosphere. The underwater PFDs at a depth of 0.1, 1, and 10 m are shown using red, green, and blue lines, respectively.

II. Evolution form water to land under M-star radiation

The adaptive evolution of phototrophs from water to land may eventually also use NIR radiation, by one of two photochemical reaction centers, with the other center continuing to use visible light. These "two-color" reaction centers can absorb more photons, but they will encounter difficulty in adapting to drastically changing light quality and quantity at the boundary between land and water (Figure 2). NIR photosynthesis can be more productive on land, though its evolution would be preceded by the Earth-type vegetation. Thus, the red-edge position caused by photosynthetic organisms on habitable M-dwarf exoplanets could initially be similar to that on Earth. Our studies imply that red-edge is a universal biosignature for life originated underwater.

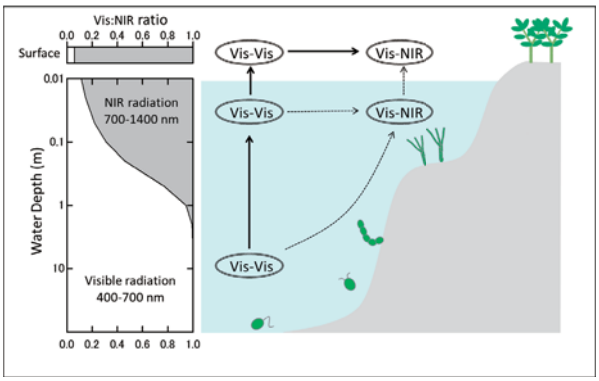

Figure 2. Lighting conditions on a hypothetical habitable planet around an M-dwarf and the evolution of photosynthesis. Ovals and arrows outline the flow of evolutionary paths from a two-photon reaction using visible radiation (Vis-Vis) to a two-color reaction using visible and NIR radiation in separate reaction centers (Vis-NIR). The area graph on the left side shows the visible-radiation/NIR-radiation ratio on the land surface and underwater at different depths.

Publication List:

〔Original paper〕

● Takizawa, K., Minagawa, J., Tamura, M., Kusakabe, N., and Narita, N. (2017). Red-edge position of habitable exoplanets around M-dwarfs. Sci. Rep. 7, 7561.

NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities were launched in 2010 to support basic biology research in NIBB. They consist of three facilities that are developing and providing state-of-the-art technologies to understand biological functions through functional genomics, bioimaging, and bioinformatics. The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among the researchers of NIBB and other academic institutions.

Functional Genomics Facility

Specially Appointed Associate Professor:
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
	BINO, Takahiro
Technical Assistant:	*ASAO, Hisayo*
	AKITA, Asaka
	MATSUMOTO, Miwako
Secretary:	*ICHIKAWA, Mariko*

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

We recently largely renovated the building of the Functional Genomics Facility. For example, the Visitors Lab and the Visitors Office were newly designed so that visiting scientists can work effectively during their stay. In 2017, approximately 200 researchers visited to use our new facility and developed active collaborations, which resulted in 16 co-authored papers published.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing HiSeq2500, HiSeq1500, and MiSeq (Illumina), and PacBio RS II (PacificBio Sciences), the Functional Genomics Facility is committed to joint research aiming to explore otherwise inaccessible new fields in basic biology.

During 2017 we carried out 56 NGS projects in collaboration with NIBB laboratories as well as the researchers of other academic institutions. These projects cover a wide range of species (bacteria, animals, plants, and humans) including both model and non-model organisms, and various applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

Figure 1. Next-generation sequencer

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2017, we analyzed approximately 750 samples with mass spectrometers and protein sequencers.

- LC-MS (AB SCIEX TripleTOF 5600 system)
- LC-MS (Thermo Fisher SCIENTIFIC Orbtrap Elite)
- MALDI-TOF-MS (Bruker Daltonics REFLEX III)
- LC-Q-TOF MS (Waters Q-TOF Premier)
- Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

Other analytical instruments (excerpts)

- Cell sorter (SONY SH800)
- Bioimaging Analyzer (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser Capture Microdissection System (Arcturus XT)
- DNA Sequencer (ABI PRISM 310; ABI 3130xl)
- Real Time PCR (ABI 7500)
- Ultra Centrifuge (Beckman XL-80XP etc.)

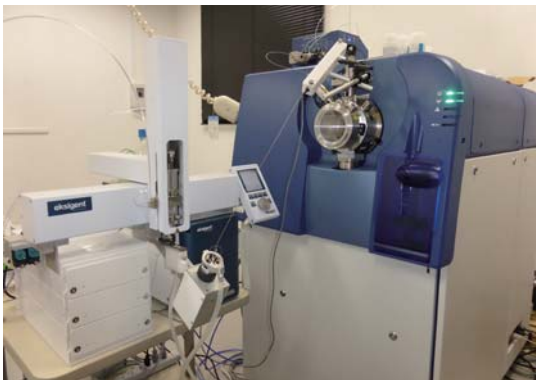

Figure 2. Triple TOF LC/MS/MS System

Publication List on Cooperation:

〔Original papers〕

- Anbutsu, H., Moriyama, M., Nikoh, N., Hosokawa, T., Futahashi, R., Tanahashi, M., Meng, X.Y., Kuriwada, T., Mori, N., Oshima, K., Hattori, M., Fulie, M., Satoh, N., Maeda, T., Shigenobu, S., Koga, R., Fukatsu, T. (2017). Small genome symbiont underlies cuticle hardness in beetles. Proc. Natl. Acad. Sci. USA *114*, E8382–E8391.
- Fukushima, K., Fang, X., Alvarez-Ponce, D., Cai, H., Carretero-Paulet, L., Chen, C., Chang, T.H., Farr, K.M., Fujita, T., Hiwatashi, Y., Hoshi, Y., Imai, T., Kasahara, M., Librado, P., Mao, L., Mori, H., Nishiyama, T., Nozawa, M., Palfalvi, G., Pollard, S.T. Rozas, J., Sanchez-Gracia, A., Sankoff, D., Shibata, T.F., Shigenobu, S., Sumikawa, N., Uzawa, T., Xie, M., Zheng, C., Pollock, D.D., Albert, V.A., Li, S., Hasebe, M. (2017). Genome of the pitcher plant Cephalotus reveals genetic changes associated with carnivory. Nat. Ecol. Evol. *1*, 59.
- Gotoh, A., Shigenobu, S., Yamaguchi, K., Kobayashi, S., Ito, F., and Tsuji, K. (2017). Transcriptome characterization of male accessory glands in ants to identify molecules involved in their reproductive success. Insect. Mol. Biol. *32*, 382.
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- Ihara, K., Sato, K., Hori, H., Makino, Y., Shigenobu, S., Ando, T., Isogai, E., and Yoneyama, H. (2017). Expression of the *alaE* gene is positively regulated by the global regulator Lrp in response to intracellular accumulation of l-alanine in *Escherichia coli*. J. Biosci. Bioeng. *123*, 444–450.
- Kondo, S., Wakae, K., Wakisaka, N., Nakanishi, Y., Ishikawa, K., Komori, T., Moriyama-Kita, M., Endo, K., Murono, S., Wang, Z., Kitamura, K., Nishiyama, T., Yamaguchi, K., Shigenobu, S., Muramatsu, M., Yoshizaki, T. (2017). APOBEC3A associates with human papillomavirus genome integration in oropharyngeal cancers. Oncogene. *36*, 1687-1697.
- Kudo, A., Shigenobu, S., Kadota, K., Nozawa, M., Shibata, T.F., Ishikawa, Y., and Matsuo, T. (2017). Comparative analysis of the brain transcriptome in a hyper-aggressive fruit fly, *Drosophila prolongata*. Insect. Biochem. Mol. Biol. *82*, 11–20.
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- Suetsugu, K., Yamato, M., Miura, C., Yamaguchi, K., Takahashi, K., Ida, Y., Shigenobu, S., and Kaminaka, H. (2017). Comparison of green and albino individuals of the partially mycoheterotrophic orchid *Epipactis helleborine* on molecular identities of mycorrhizal fungi, nutritional modes, and gene expression in mycorrhizal roots. Mol. Ecol. *26*, 1652-1669.

Specially Appointed Associate Professor: *SHIGENOBU, Shuji*
NIBB Research Fellow: *OGAWA, Kota*
Visiting Graduate Student: *HSIAO, Yi-Min*
Technical Assistant: *SUZUKI, Miyuzu*

Symbiogenomics

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

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

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

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

bacteriocytes.

We recently discovered a novel class of genes in the pea aphid genome that encode small cysteine-rich proteins with secretion signals that are expressed exclusively in bacteriocytes of the pea aphid, and named these bacteriocyte-specific cysteine-rich proteins (BCR). The BCR mRNAs are first expressed at a developmental time point coincident with the incorporation of symbionts strictly in the cells that contribute to the bacteriocyte, and this bacteriocyte-specific expression is maintained throughout the aphid's life. Some BCRs showed an antibiotic activity. These results suggest that BCRs act within bacteriocytes to mediate the symbiosis with bacterial symbionts, which is reminiscent of the cysteine-rich secreted proteins of leguminous plants that also regulate endosymbionts. Employment of small cysteine-rich peptides may be a common tactic of host eukaryotes to manipulate bacterial symbionts.

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

Publication List:

〔Original papers〕

- Anbutsu, H., Moriyama, M., Nikoh, N., Hosokawa, T., Futahashi, R., Tanahashi, M., Meng, X.-Y., Kuriwada, T., Mori, N., Oshima, K., Shigenobu, S., *et al*. (2017). Small genome symbiont underlies cuticle hardness in beetles. Proc. Natl. Acad. Sci. USA *114*, E8382–E8391.
- Hayashi, M., Shinozuka, Y., Shigenobu, S., Sato, M., Sugimoto, M., Ito, S., Abe, K., and Kobayashi, S. (2017). Conserved role of Ovo in germline development in mouse and *Drosophila*. Sci. Rep. *7*, 40056.
- Ota, R., Morita, S., Sato, M., Shigenobu, S., Hayashi, M., and Kobayashi, S. (2017). Transcripts immunoprecipitated with Sxl protein in primordial germ cells of *Drosophila* embryos. Dev. Growth Differ. *59*, 713–723.

〔Review article〕

- Mergaert, P., Kikuchi, Y., Shigenobu, S., and Nowack, E.C.M. (2017). Metabolic integration of bacterial endosymbionts through antimicrobial peptides. Trends Microbiol. *25*, 703–712.

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor:
KAMEI, Yasuhiro

Technical Staff: *KONDO, Maki*
TANIGUCHI-SAIDA, Misako
Technical Assistant: *ICHIKAWA, Chiaki*
ISHIKAWA, Azusa

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

Representative Instruments: Okazaki Large Spectrograph (OLS)

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

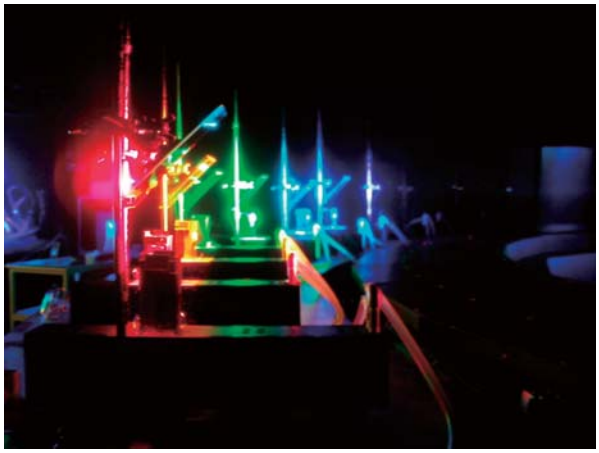

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

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

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

Microscopes

This facility also has Bioimaging machines such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Olympus FV1000, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD camera), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced laser microscopes with special aims (Light-sheet Microscope and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began two new types of Collaborative Research Program from 2016. One is a new category of the NIBB Collaborative Research for Integrative Bioimaging using machines and bioimage processing/analysis techniques, and the other is the Advanced Bioimaging Support Program (ABiS) of the Grant-in-aid for Scientific Research on Innovative Areas.

The light-sheet microscope was developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissue by illuminating a specimen from the side with a light sheet (more information is given in Dr. Nonaka's section: Lab. for Spatiotemporal Regulations). Dr. Shigenori Nonaka conducted and supported about 10 projects as Collaborative Research Programs for Integrative Bioimaging. The IR-LEGO was developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST). This microscope can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser. Details are described in the next section. The IR-LEGO was also used for about 10 Collaborative Research projects, including applications for animals and plants.

Workshop and Symposium

In 2017 we held the 5th biological image processing training course with the Department of Imaging Science, CNSI. We also have been holding a "Bioimaging Forum" every year which discusses Bioimaging from various directions such as microscopy, new photo-technology, and computer science. This year we held the 11th NIBB Bioimaging Forum focused on adaptive optics for microscopy. In addition, we held three symposiums focused on new emerging model animals, next generation research using amphibians, and Biothermology: heat and temperature in biology.

Publication List on Cooperation

〔**Original papers (Selected)**〕

● Homma, N., Harada, Y., Uchikawa, T., Kamei, Y., and Fukamachi, S.

(2017). Protanopia (red color-blindness) in medaka: a simple system for producing color-blind fish and testing their spectral sensitivity. BMC Genetics *18*, 10.
- Ishikawa, T., Kashima, M., Nagano, A.J., Ishikawa-Fujiwara, T., Kamei, Y., Todo T., and Mori, K. (2017). Unfolded protein response transducer IRE1-mediated signaling independent of XBP1 mRNA splicing is not required for growth and development of medaka fish. eLife *6*, e26845.
- Li, C., Sako, Y., Imai, A., Nishiyama, T., Thompson, K., Kubo, M., Hiwatashi, Y., Kabeya, Y., Karlson, D., Wu, S. H., Ishikawa, M., Murata, T., Benfey, P.N., Sato, Y., Tamada, Y., and Hasebe, M. (2017). A Lin28 homologue reprograms differentiated cells to stem cells in the moss Physcomitrella patens. Nat. Commun. *8*, 14242.
- Nakano, M., Arai, Y., Kotera, I., Okabe, K., Kamei, Y., and Nagai, T. (2017). Genetically encoded ratiometric fluorescent thermometer with wide range and rapid response. PLoS ONE *12*, e0172344.
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- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M., Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Commun. *8*, 412.
- Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Campbell, R.E., and Ueno, N. (2017). Distinct intracellular Ca2+ dynamics regulate apical constriction and differentially contribute to neural tube closure. Development. *144*, 1307-1316.
- Taniguchi, A., Kimura, Y., Mori, I., Nonaka, S., and Higashijima, S.-I. (2017). Axially-confined in vivo single-cell labeling by primed conversion using blue and red lasers with conventional confocal microscopes. Dev. Growth Differ. *59*, 741-748.

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

- Nakamoto, M., Shibata, Y., Ohno, K.. Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. 2017 July 13.

● *Research activity by Y. Kamei*

Specially Appointed Associate Professor:	*KAMEI, Yasuhiro*
NIBB Research Fellow:	*SAKAMOTO, Joe*
Visiting Scientist:	*HATTORI, Masayuki*
Technical Assistant:	*NAKAGAWA, Mami*

Our research group promotes two cutting-edge microscope projects; "observation" and "manipulation" using optical and biological technologies. The aim of our "observation project" is deep-seeing in living organisms using adaptive optics (AO) which were well-developed in the field of astronomy as a key technology of large telescopes such as the Subaru telescope in Hawaii. Although observation using telescopes on the earth may be disturbed by fluctuations in the atmosphere, AO technology can cancel this disturbance. On the other hand, living materials have particular refractive

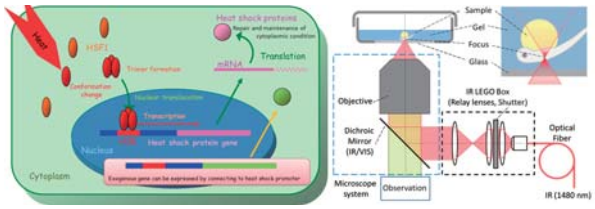


Figure 1. Schematic illustration of heat shock response of cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

indexes, therefore, some organelles act as disturbances of the ideal optical path for microscope observation just like the atmosphere does for telescopes. AO technology can also compensate for this disturbance by sensing and correcting wave fronts using a wave front sensor and deformable mirror. Hence, we developed a custom-made wide-field microscope equipped with an AO system for observation of living organisms in collaboration with Dr. Tamada in NIBB and Dr. Hayano in the National Astronomical Observatory of Japan (NAOJ) and got high-resolution bright field and fluorescent images of living cells. Our results indicated that improvement of optical resolution was restricted to a small area which is called the "isoplanatic patch".

Second, the aim of our "manipulation project" is to control gene expression *in vivo*. Gene function analysis must be evaluated at the cell level *in vivo*. To achieve spatiotemporal-controlled gene expression we employed one of the stress responses, the heat shock response. The heat shock promoter is the transcription regulation region of heat shock proteins and all organisms have this mechanism. Positioning the target gene downstream of the promoter, we can induce the target gene expression by local heating.

Infrared (IR) beams can heat water molecules, which are the main constituent of cells, hence, we can heat a single cell by irradiating IR to a target cell using a microscope. We have developed a microscope, IR laser evoked gene operator (IR-LEGO), specialized for this purpose (Figure 1). The IR-LEGO microscope can irradiate an IR laser to a single cell *in vivo* such as in *C. elegans*, *Drosophila*, medaka, zebrafish, *Xenopus* and *Arabidopsis*, to induce the heat shock response at a desired timing. In 2015, additionally, we confirmed the system was effective in the moss *Marchantia polymorpha* and in the newt *Pleurodeles waltl*.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating. We evaluated time course and spatial heating profiles, and the results presented that temperature of the target area rose rapidly and kept a constant level dependant on IR laser power, additionally, the heated area was adequately as small as a typical cell size.

With this in mind, we tried to induce gene expression in various species. At first, we reported an IR-LEGO experiment in living *C. elegans*. Target gene expression in a target cell could be induced with only 1 s-IR irradiation. Whereas the optimal power range which can induce gene induction without cell damage was limited. Excess laser power resulted in cell death or cessation of cell division. We confirmed that an optimal irradiation, e.g. 11 mW for 1 s, induced physiological gene expression in the target cell and subsequent

cell division or morphogenesis underwent normal development. Next, we tried the experiment in other animals, such as, medaka, zebrafish and *Xenopus*, and the higher plant, *Arabidopsis*, since all organisms have a heat shock response system. We succeeded in local gene induction in all the species as expected. Moreover, this system can be combined to the cre/loxP recombination technique for long-term gene expression (Figure 2).

As mentioned above, excess irradiation resulted in cell damage, so we utilized the system to ablate target cells with strong pulsed irradiation. In collaboration with National Taiwan University, we used the system for neuronal regeneration study in zebrafish and revealed that a kind of neuronal precursor cell played an important role within the neuron regeneration step the in zebrafish spinal cord (Zeng *et al*. Biol. Cell 2016). In addition, the IR-LEGO system can be utilized for biothermology, a new field thinking about temperature or heat in biological systems, because spatiotemporal micrometer order local heating is difficult without this system. Now we are trying to estimate thermal properties of cells and biomaterials *in vivo* using a newly developed thermo-probe (Nakano *et al*. PLoS One 2017) and the IR-LEGO microscope system.

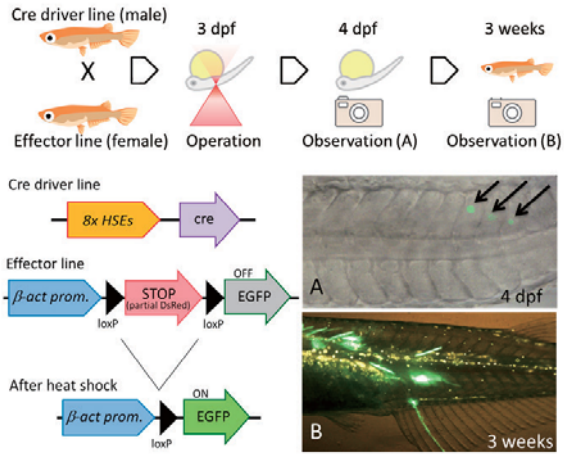


Figure 2. Long-term gene expression system with cre/loxP recombination system and its example of practical experiment in medaka embryo to adult.

Publication List:

〔Original papers〕

- Hattori M., Tamada Y., Murata T., Oya S., Hasebe M., Hayano Y., and Kamei Y. (2017). Artificial testing targets with controllable blur for adaptive optics microscopes. Optical Engineering *56*, 080502.
- Homma, N., Harada, Y., Uchikawa, T., Kamei, Y., and Fukamachi, S. (2017). Protanopia (red color-blindness) in medaka: a simple system for producing color-blind fish and testing their spectral sensitivity. BMC Genetics *18*, 10.
- Ishikawa, T., Kashima, M., Nagano, A.J., Ishikawa-Fujiwara, T., Kamei, Y,, Todo T., and Mori, K. (2017). Unfolded protein response transducer IRE1-mediated signaling independent of XBP1 mRNA splicing is not required for growth and development of medaka fish. eLife *6*, e26845.
- Ishikawa, T., Toyama, T., Nakamura, Y., Tamada, K., Shimizu, H., Ninagawa, S., Okada, T., Kamei, Y., Ishikawa-Fujiwara, T., Todo, T., Aoyama, E., Takigawa, M., Harada, A., and Mori, K. (2017). UPR transducer BBF2H7 allows export of type II collagen in a cargo- and developmental stage-specific manner. J. Cell Biol. *216*, 1761-1774.
- Nakano, M., Arai, Y., Kotera, I., Okabe, K., Kamei, Y., and Nagai, T. (2017). Genetically encoded ratiometric fluorescent thermometer with wide range and rapid response. PLoS ONE *12*, e0172344.
- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M., Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Commun. *8*, 412.

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

- Nakamoto, M., Shibata, Y., Ohno, K.. Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. 2017 July 13.

Data Integration and Analysis Facility

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

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

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a high-performance cluster system (SGI Rackable server C2112-4RP; 40 nodes/800 cores, 96GB memory/node), a shared memory parallel computer (HP ProLiant DL980 G7; 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700; 480TB), and a large capacity storage system (DELL PowerEdge R620; 720TB). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be processed efficiently. Some personal computers and color 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 and collaborative researchers. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

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

Figure 1. Biological Information Analysis System

Research activity by I. Uchiyama

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p. 69).

NIBB BioResource Center

Head
FUJIMORI, Toshihiko

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

Model Animal Research Facility

Associate Professor:	*WATANABE, Eiji*
Technical Staff:	*OHSAWA, Sonoko*
	HAYASHI, Kohji
	NOGUCHI, Yuji
Technical Assistant:	*TAKAGI, Yukari*
	SUGINAGA, Tomomi
	FUJIMOTO, Daiji
	TAKAHASHI, Nobuaki
	MATSUMURA, Kunihiro
	NOMOTO, Yoshihiro

Figure 1. Mouse breeding room in the Yamate area

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

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

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

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

1. The provision of information, materials, techniques, and animal housing space to researchers.
2. The use of various kinds of instruments to analyze mutant, transgenic, and gene-targeted animals.
3. The development of novel techniques related to transgenic and gene targeting technology.
4. Cryopreservation and storage of transgenic mice strains.
5. Generating genetically-engineered mice using the CRISPR/Cas9 method.

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

Figure 2. Large sized autoclave in the Yamate area.

In 2017 (from January 1 to December 21), 3,940 mice (7 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 40,428 mice (including pups bred in the facility) were taken out.

A number of strains of genetically altered mice from outside

the facility were brought into the mouse housing area by microbiological cleaning using *in vitro* fertilization-embryo transfer techniques (12 transgenic lines), and stored using cryopreservation (28 transgenic lines). Frozen eggs of 33 mice lines were taken out of the facility.

Genome editing experiments were performed on three kinds of target genes. We generated gRNAs of the target genes, which were transferred into fertilized eggs with Cas9 protein. We were able to introduce intended mutations into the genome DNA.

A new mouse facility in the Myodaiji area opened at the beginning of 2005. The facility provides research-supporting activities to researchers in the Myodaiji area. In March 2009, we expanded the facility which includes areas for breeding, behavioral tests, and transgenic studies using various kinds of recombinant viruses. In 2017 (from January 1 to December 21), 0 mice were brought into the facility in the Myodaiji area, and 1,246 mice (including pups bred in the facility) were taken out.

Figure 3. Equipment for gene transfer.

II. Research support activities (small fish and birds)

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

In 2017 (from January 1 to December 21), 75 zebrafish (75 fertilized eggs) were brought to the facility. In the laboratory for chick embryos there were no fertilized eggs or chicken embryos brought in or taken out this year. These animals were used for research activities in neurobiology and developmental biology.

Figure 4. Liquid nitrogen tank.

III. Research activities

The associate professor of this center - E. Watanabe - is the principal investigators of the Laboratory of Neurophysiology. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. For details, please refer to the page of the laboratory (p. 44).

Model Plant Research Facility

● ***Plant Culture Laboratory***

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

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

The Plant Culture Laboratory equips and manages 73 culture boxes or growth chambers, and 13 rooms with the P1P physical containment level for established and emerging model plants, for example the thale cress *Arabidopsis thaliana*, the rice *Oryza sativa*, the moss *Physcomitrella patens*, the liverwort *Marchantia polymorpha*, the green alga *Chlamydomonas reinhardtii* and several other flowering plants including several carnivorous plants. Most culture space is fully used the whole year by more than 70 researchers from both outside and inside groups.

As well as regular culture conditions, extreme environmental conditions for light and temperature are available for various types of experiments. Three chambers (3.4 m^2 each) that can control CO_2 and humidity in addition to temperature and light (max 70,000 lux) conditions are available. A tissue culture rack with dimming LEDs and pulse-width modulation controllers are used for algae culture under precise light control. Autotrophic and heterotrophic culture devices are

also available for researchers using cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches and a safety cabinet. Several analytical instruments including a flow cytometry system and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available.

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

The building renovation work was completed in May 2017, and the temporally closed facilities including the three chambers that can produce extreme environmental conditions were renewed and opened.

Cell Biology Research Facility

Associate Professor: *WATANABE, Eiji*

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

Figure 5. Equipment for tissue and cell culture.

NATIONAL BIORESOURCE PROJECT

The major purpose of the National BioResource Project (NBRP) is to collect, preserve, and provide bioresources (such as experimental animals and plants) that are essential experimental materials for life sciences research. The project also aims to improve the bioresources by increasing their value through enriching genome information and developing fundamental technologies for preservation and other necessary procedures, in order to meet to the current scientific demands. NIBB serves as the core organization center of the medaka bioresource and as sub-centers of the morning glory and the zebrafish bioresources.

I. NBRP Medaka (*Oryzias latipes*)

Project Manager: *NARUSE, Kiyoshi*
Sub Managers: *KAMEI, Yasuhiro*
ANSAI, Satoshi

NBRP medaka provides three groups of resource worldwide, including 1) live medaka resources comprising more than 600 strains (strains for general use, wild populations, related species, inbred strains, mutants, and transgenics), 2) genome resources (ca. 400 thousand cDNA clones originated from 33 cDNA libraries, and BAC/Fosmid clones covering the whole medaka genome), and 3) hatching enzyme necessary for manipulation and live imaging of the medaka embryo. Entries for these resources can be searched by various methods such as keywords, sequence homology, and expression profile on the web site (https://shigen.nig.ac.jp/medaka/).

We provide mutant gene screening using the HRM method in the TILLING library, and also provide a genome editing platform using CRISPR/Cas9. Using collaborative research support, researchers can visit NIBB to generate mutants by genome editing. We developed and provided a new genome browser (http://viewer.shigen.info/medaka/index.php) using a recently published genome assembly by PacBioRSII.

Summary of previous accomplishments are as follows. In 2007-2009, we sequenced both ends of 260,000 clones originated from 11 kinds of full-length cDNA libraries and sequenced the whole length of 17,000 independent clones. We also developed strains in which CRE-recombinase can be expressed in any cell lineages using a heat shock promoter, and started to provide strains (TG918, TG921, etc) established using this method. In 2010, we re-sequenced the genomes of five inbred strains by coverage corresponding to genome 100X (http://medaka.lab.nig.ac.jp/service/menu). In 2012, we developed a vitrification freezing preservation method of the testicular tissue. We are now providing a backup preservation service of testicular tissues using this method.

NBRP Medaka fish facility to collect, maintain and supply the five medaka.

II. NBRP Morning Glory (*Ipomoea nil*)

Project Manager: HOSHINO, Atsushi

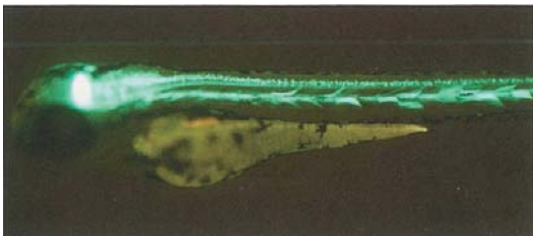
The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, and is studied worldwide, especially in plant physiology and genetics. NIBB collects, develops and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning Glory, and collaborates with the core organization center, Kyushu University. We collected several mutant lines, and provided 122 mutant lines and 4 DNA clones to both local and international biologists in 2017. A database for the whole genome sequence of *I. nil* was updated.

Various colors of morning glory flowers. The color depends not only on the molecular structure of the pigment, but also on the pH of the vacuole where the pigment is accumulated.

III. NBRP Zebrafish (*Danio rerio*)

Project Manager: HIGASHIJIMA, Shin-ichi

NIBB is a sub-center of the National BioResource Project (NBRP) Zebrafish, and collaborates with the core organization center, RIKEN Brain Science Institute. We at NIBB mainly collect zebrafish strains expressing fluorescent proteins in specific cells of the central nervous system and distribute them to researchers worldwide. Zebrafish is an important and globally used experimental vertebrate model animal with a simple body structure, it allows genetic manipulations, and its embryos are transparent enough for optical observations. Researches using zebrafish for the studies of neural development and neural circuit functions are growing rapidly worldwide, and the importance of strains collected and provided by NIBB to researchers is growing accordingly.



An example of transgenic fish generated by the CRISPR/Cas9-mediated knock-in method.

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

Specially Appointed Professor
NARUSE, Kiyoshi

Specially Appointed Assistant Professor: *TAKETSURU, Hiroaki*
Specially Appointed Senior Specialist: *AKIMOTO-KATO, Ai*
Technical Assistant: *MATSUBAYASHI, Naomi*
MIZOKAMI, Yuko
TSUZUKI, Chizuru

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

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

Figure 1. IBBP Center.

Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

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

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

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

I. Current status of back up for the biological resources

In 2017, IBBP Center stored 4,478 384-well and 69 96-well plates consisting of 1,726,176 clones as cDNA/BAC clones, 12,225 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 1769 133mm-straw tubes for sperm and 654 seed samples. In total 1,728,599 samples are stored.

Figure 3. Cryotube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

II. Collaborative Research Project for the development of new long-term storage technologies and cryo-biological study

As the IBBP Center can only accept biological resources which can be cryopreserved in liquid nitrogen, researchers cannot backup those biological resources for which cryo-preservation methods are not well established, to increase the usability of IBBP, we started a collaborative research project for the development of new long-term storage technologies and cryo-biological study in 2013. This collaborative research includes two subjects 1) Establishment of new storage technologies for biological resources for which long-term storage is unavailable. 2) Basic cryobiological research enabling us to improve low temperature storage for biological resources. In 2017, we also worked to establish a research center for cryo-biological studies thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2017 on November 1-2, 2017 at the Tsukuba Center for Institutes, Tsukuba, Ibaraki, Japan. We had 132 participants from several fields covering physics, chemistry, biology, and technology.

Figure 4. Group photo of Cryopreservation Conference 2017.

Research activity by H. Taketsuru

Specially Appointed Assistant Professor:
TAKETSURU, Hiroaki

Development of rat vitrified embryos in each developmental stage

Vitrification of embryos is a simple method and is used in various animals. Although vitrification of 2-cell embryos is possible, only a few reports were published about vitrification of embryos in various developmental stages. Vitrified pronuclear and morula stage embryos are used for producing genetically engineered rats and knockout/in rats. This study examined the development of rat embryos in pronuclear, 2-cell and morula stages after vitrification.

Wistar female rats aged more than 8 weeks were injected with 150 IU/kg PMSG and 75 IU/kg hCG. Females were then mated with mature male rats of the same strain. Pronuclear and 2-cell stage embryos were collected from oviducts. Morula embryos were collected from the uterus. Pronuclear, 2-cell and morula stage embryos were vitrified using a solution containing 10% propylene glycol, 30% ethylene glycol, 20% Percoll and 0.3 M sucrose in PB1.

Figure 5. Vitrification and transfer of rat embryos.

Each embryo was warmed using a solution containing 0.3 M sucrose in PB1. Morphologically normal embryos were transferred into the oviducts of the pseudopregnant females (Figure 5). Pronuclear embryos were vitrified after culturing for 0, 4 and 7 hours. After warming, the embryos that developed to the 2-cell stage were transferred into the oviducts of the pseudopregnant females.

The development to offspring of vitrified-warmed pronuclear, 2-cell and morula stage embryos were 16%, 33% and 52%, respectively (Figure 6). When the pronuclear embryos were vitrified after culturing for 0, 4 and 7 hours, the developmental rates to offspring were 12%, 25% and 24%, respectively. These results indicated that tolerance to vitrification was increased with embryo development. Vitrified embryos can be used for producing genetically engineered rats and knockout/in rats.

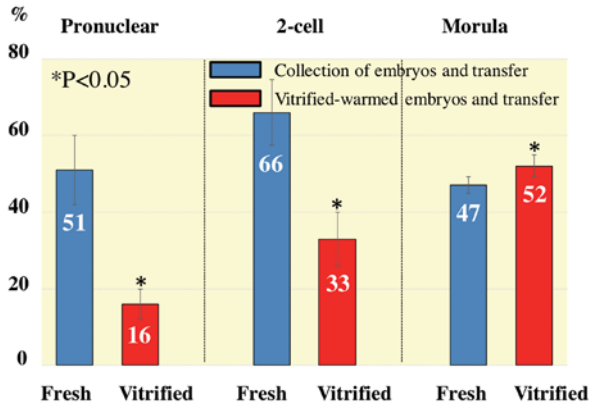


Figure 6. Development to offspring of vitrified embryos.

The level of ovulation in various rat strains and development of embryos after vitrification

In rats, it is now possible to produce genetically engineered strains, not only as transgenic animals but also using gene knockout techniques. Reproductive technologies have been used as indispensable tools to produce and maintain these novel valuable strains. Here we studied the sensitivity to PMSG/hCG for oocyte collection, and the development of embryos after cryopreservation in some strain rats (Figure 7).

Figure 7. Rat strains.

The response to pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) was examined by injection of 150IU/kg PMSG + 75IU/kg hCG or 300IU/kg PMSG + 300IU/kg hCG. The numbers of 2-cell embryos collected from F344/Stm female rats were high (31.0). On the other hand, the number of 2-cell embryos collected from BN/SsNSlc female rats were low (2.4) in these strains. Of cryopreserved 2-cell embryos collected from these strains, more than 65% of embryos survived after warming. After transfer, offspring were obtained from cryopreserved 2-cell embryos in all strains. However, the offspring rate was different for strains, F344/Stm and Jcl: Wistar were more than 50%, and ACI/Nkyo was 20% (Figure 8).

In summary, there are differences in sensitivity to superovulation and the development to offspring rate from cryopreserved embryos in these strains.

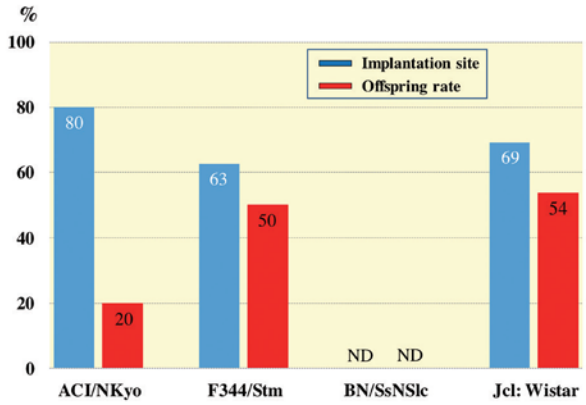


Figure 8. Development to offspring of vitrified embryos in some strains.

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor
KODAMA, Ryuji

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

Technical Assistant: *HAYASHI, Tomoko*

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

Ms. Matsuda, Ms. Iinuma and Ms. Hayashi maintained the Myodaiji area. Ms. Sawada worked in the Yamate area. Dr. Kodama worked in both areas.

The following are the CRF's notable activities in 2017.

1. The CRF usage charge system was started.

At April 2017, the users of the CRF must pay the facility usage charge which equals the disposal cost of radioactive wastes generated in the user's experiment or in the decontamination processes of the contamination apparently caused by the user. The CRF prepared each laboratory's radioactive waste container. (Figure 1A)

2. The "User's guide" in English was renewed.

Main points changed were the explanation and the documents about the CRF usage charge.

3. The CRF website was renewed.

The main point changed was the usage of new web design software, which allows users to access the contents more conveniently http://www.nibb.ac.jp/ricenter/ (Figure 1B)

The number of registrants and the number of users from January 2017 to December 2017 are shown in Table 1.

Users and visitors counted by the access control system of the controlled areas numbered 1,302 during this period. The numbers for each area are shown in Table 2. The annual changes of registrants and the number of totals per fiscal year are shown in Figure 2. The balance of radioisotopes received and used at the CRF is shown in Table 3. Yamate area storage radioisotopes were transferred to the storage room in Myodaiji area for saving energy at Summer 2017. As the result, radioisotopes received apparently increased. But the new radioisotope received and radioisotope experimental handling user was only 1 at the Myodaiji-area from April 2017. The training courses on radioisotope handling were given as in Table 4.

Figure 1. The CRF's notable activities in 2017.
A: the radioactive waste containers for each laboratory.
B: renewal of website of the CRF

	Myodaiji Area	Yamate Area
Registrants	47	43
Users	21	24

Table 1. Numbers of registrants and users at Myodaiji area and Yamate area in 2017.

	Myodaiji Area	Yamate Area	Total
Users	527	488	1,015
Visitors	125	162	287
Total	652	650	1,302

Table 2. Users and visitors who entered each controlled area in 2017.

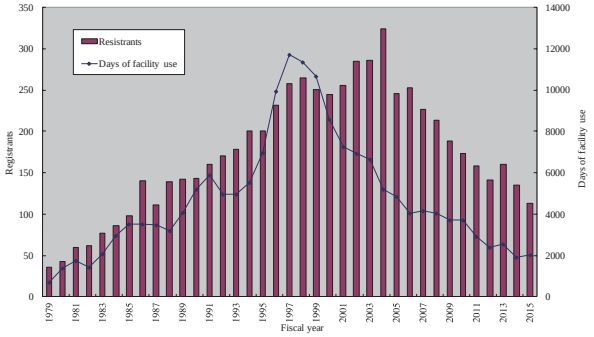


Figure 2. Annual changes of registrants and days of facility use per fiscal year.

	Myodaiji Area	Yamate Area	Total
^{125}I Received	111	221	332
^{125}I Used	0	3,329	3,329
^{35}S Received	73,186	0	73,186
^{35}S Used	0	25,438	25,438
^{32}P Received	9,250	9,250	18,500
^{32}P Used	9,250	9,250	18,500
^{14}C Received	3,913	3,913	7,827
^{14}C Used	2,079	0	2,079
^{3}H Received	676,104	676,104	1,352,209
^{3}H Used	1,480	933,917	935,397

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2017.

training course	place	numbers of particip
Introductory course for beginners	Myodaiji	1
Introductory course for beginners	Yamate	1
Introductory course for experts	Myodaiji	2
Introductory course for experts	Yamate	2
Users training course*	Myodaiji	39
Users training course	Yamate	31

*including English course

Table 4. Training courses for radiation workers in 2017.

RESEARCH ENHANCEMENT STRATEGY OFFICE

Director
UENO, Naoto

Vice-Director:
MANO, Shoji (Nov. 1~)

Vice-Director: NISHIMURA, Mikio ~Sep. 30)

In order to fulfill two goals, to encourage cutting-edge academic research in the field of natural sciences through international joint research, and to contribute to the enhancement of research capabilities of universities etc. in Japan using the world's most advanced research environment for joint utilization and joint research, NINS started in 2013 a research enhancement project with the following four approaches: 1) Support for the promotion of international advanced research, 2) Support for the promotion of joint utilization and joint research in Japan, 3) Dissemination of information and enhancement of public relations in Japan and abroad, 4) Support for researchers, especially young, female or foreign researchers.

The Research Enhancement Strategy Office is aimed at supporting researchers so that NIBB improves its ability as a collaborative research institution, and was restructured in 2013 from the former Strategic Planning Department, the Office of Public Relations, and the Office of International Cooperation which existed from 2005. The Office's activities are mainly carried out by URAs (University Research Administrators) according to the advice of the group adviser chosen from NIBB's professors and in close collaboration with the NINS's Research Enhancement Promotion Headquarters.

In 2017, Young Researcher Support Group was newly added as the 6th group to the Research Enhancement Strategy Office to provide comprehensive support for young researchers in NIBB.

Evaluation and Information Group

Associate Professor: *KODAMA, Ryuji*
Group Adviser: *KAWAGUCHI, Masayoshi*

This group serves as a central office for assisting the Director General in preparing for NIBB's evaluation procedure and in planning long-range strategies for the institute.

The main activities of the group

1) Management of external evaluation processes

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

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

This group edits the annual report (this print) which summarizes the annual activities of laboratories and facilities and is used in the evaluation procedures of the institute.

3) Assistance in budget requests and long-range planning of the institute

This group also assists the Director General in preparing long-range plans for building the most advanced research facilities, and in budget requests to the government to realize and implement these plans.

4) Assistance in making the plans and reports of the institute

In addition we assist in drafting NIBB's Medium-term Goals and Plans (for a six-year-period), and in instituting Annual Plans to implement them. The department also assists in preparing Business and Performance Reports for external evaluation on whether we are meeting the goals set both annually and for the medium-term.

Public Relations Group

Specially Appointed Assistant Professor (URA): *KURATA, Tomoko*
Technical Assistant: *OTA, Kyoko*
KAWAGUCHI, Colin
BAN, Misato
Group Adviser: *FUJIMORI, Toshihiko*

This group, in order to publicize the activities of NIBB to the widest audience, actively facilitates communication between NIBB and the public, school teachers, and the international community of scientific researchers.

The main activities of the group in 2017

1) Press releases

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

2) Updating and maintenance of the NIBB web page

3) Editing of publications, production of posters and leaflets

Publication of "NIBB News" (Intra-institutional newsletter, in Japanese). Publication of leaflets introducing the individual researchers of NIBB (in Japanese). Design and distribution of posters of NIBB events.

4) Producing Videos

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

5) Organization of scientific outreach programs

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

INTERNATIONAL COOPERATION GROUP

Specially Appointed Assistant Professor (URA): *TATEMATSU, Kiyoshi*
Technical Assistant: *TAKAHASHI, Ritsue*
KAWAGUCHI, Colin
NISHIMURA, Akiko
Group Advisor: *UENO, Naoto*

NIBB has a mission to continually explore the leading-edge of biology and form research communities that link Japan to the world. For this purpose, NIBB holds scientific meetings including "NIBB Conferences" and "Okazaki Biology Conferences (OBC)", and educational programs such as "NIBB International Practical Courses". Further, NIBB is tightly interacting with the European Molecular Biology Laboratory (EMBL, European member states), the Temasek Life Sciences Laboratory (TLL, Singapore) and Princeton University (USA) on the basis of cooperative agreements, through exchanging people and techniques and jointly holding scientific meetings. NIBB is also conducting the "NIBB International Collaborative Research Initiative" to promote high-level international collaborations between faculty members of NIBB and researchers around the world. NIBB invites leading-edge researchers from abroad as "Guest Professors" to promote academic exchange with NIBB members and to start new international collaborations.

This group supports and coordinates NIBB's activities related to international research collaborations, through organizing the various above-mentioned international scientific meetings and technical courses, coordination of dispatching NIBB's researchers to international conferences, and support of researchers visiting from the institutes mentioned above. This group also supports NIBB internship students visiting from foreign countries, and the dispatching of graduate students of SOKENDAI (the Graduate University for Advanced Studies) to international conferences, which are aimed at nurturing the next generation of researchers in biology. This group, cooperating with the Okazaki Administration Office and International Affairs Division, City of Okazaki, supports other researchers and students who visit NIBB.

The main activities of the group in 2017

1) Coordination of international conferences and the International Practical Course

This group coordinated the following international conference hosted by NIBB:

- The 65th NIBB Conference "Renaissance of *Marchantia polymorpha* – the genome and beyond" Okazaki, Japan, December 16 – 18, 2017 (p. 94)

2) Support of visiting researchers to NIBB

This group supported visits of foreign researchers related to the following events:

- Two researchers coming from Princeton University as lecturers for The NIBB-Princeton Joint Proteomics Training Course (July 19-21, 2017; p. 96)
- One Dean for Research coming from Princeton University (October)

3) Support of education-related programs

This group supported graduate students participating in the following student exchange programs of NIBB/EMBL and NIBB internship:

- The 19th EMBL International Ph.D. Symposium "Bridging the Gaps – Interdisciplinary Approaches in Life Sciences –" Heidelberg, Germany, October 19-21, 2017(p. 95)
- NIBB Internship Program 2017 (p. 100)

Collaborative Research Group

Specially Appointed Associate Professor (URA): *SHIGENOBU, Shuji*
KAMEI, Yasuhiro
Technical Assistant: *ICHIKAWA, Mariko*
ICHIKAWA, Chiaki
Group Adviser: *YOSHIDA, Shosei*

Specially appointed associate professors of this group belong to the NIBB core research facilities and are responsible for managing collaborative research projects and practical courses taking advantage of their expertise in their field, through which this group explores further promotion of information exchange and collaboration among scientific communities, and also supports the development of new equipment and methods.

In 2017, this group hosted a total of 99 collaboration projects. The success of these collaborations are proved by 30 research papers published in this year. One of the examples is a 5-year collaboration of the group of Drs. Kondoh (Osaka Univ. and Kyoto Sangyo Univ.) and Shigenobu (NIBB): they established a technique of ChIP-seq using *in vivo* biotinylated transcription factors (TFs), which enabled them to reveal the gene regulatory network in mouse epiblast stem cells where ZIC2 TF plays a central role (Matsuda et al., 2017). Another noteworthy achievement is a series of publications about development of a platform to study the psychology of vision using medaka fish. Dr. Fukamachi (Japan Woman's University) conducted the Priority Collaborative Research Project "Study of molecular mechanisms for vision and color perception-dependent sexual behavior" with many collaborators including Dr. Kamei and other NIBB members. The collaboration team created a red opsin KO medaka and analyzed the red-color perception using the Okazaki Large Spectrograph (Homma et al., 2017). Dr. Watanabe (NIBB) used a 3D virtual imaging system and found that movement is crucial for medaka to discriminate each other (Nakayasu et al., 2017). Dr. Yoshimura (NIBB and Nagoya Univ.) revealed seasonal changes in color perception of medaka by using the resources and the platform developed as described above (Shimmura et al., 2017).

- Homma, N., Harada, Y., Uchikawa, T., Kamei, Y., and Fukamachi, S. (2017). Protanopia (red color-blindness) in medaka: a simple system for producing color-blind fish and testing their spectral sensitivity. BMC Genetics *18*, 10.
- Matsuda, K., Mikami, T., Oki, S., Iida, H., Andrabi, M., Boss, J.M., Yamaguchi, K., Shigenobu, S., and Kondoh, H. (2017). ChIP-seq analysis of genomic binding regions of five major transcription factors in mouse epiblast stem cells that highlights a central role for ZIC2. Development *144*, 1948-1958.
- Nakayasu, T., Yasugi, M., Shiraishi, S., Uchida, S., and Watanabe, E. (2017). Three-dimensional computer graphic animations for studying social approach behaviour in medaka fish: Effects of systematic manipulation of morphological and motion cues. PLoS ONE *12*, e0175059.
- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M., Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Commun. *8*, 412.

Young Researcher Support Group

Assistant Professor: *KOMINE, Yuriko*
Group Adviser: *FUJIMORI, Toshihiko*

Cultivation of future researchers is also an important mission of NIBB, as an international leading institute in the field of basic biology. Constituting the Department of Basic Biology in the School of Life Science of SOKENDAI (the Graduate University for Advanced Studies), NIBB provides PhD courses for graduate students. The young researcher support group was organized in 2017 aiming to support young researchers in NIBB, including graduate students (SOKENDAI students and special research students from other universities), to help make their study and research experiences in NIBB effective and fruitful for their future careers.

The main activities of this group

1) Coordination and management of courses provided by the Department of Basic Biology (lectures, research presentation, etc.), in collaboration with all faculty members in NIBB.
2) Support in organizing NIBB's programs related to the PhD courses, such as the Open Campus Day for prospective students.
3) Cooperation in the interdepartmental programs offered by the SOKENDAI headquarters, including the Freshman Course.
4) Gathering and providing information useful for both students and faculty members.

Gender Equality Promotion Group

Associate Professor: *TSUBOUCHI, Tomomi*
Group Adviser: *TAKADA, Shinji*

NIBB is committed to promoting gender equality in the scientific community. To help scientists maintain their work-life balance and progress their carriers during life events, such as having children, this group manages the research assistant system for those in need, and promotes networking among female researchers.

In 2017, this group established a mailing list for female researchers working in NIBB, held several meetings to facilitate networking and provided opportunities to discuss their concerns on career development. This group has also been working towards setting up a room where NIBB members can bring their children to work.

TECHNICAL DIVISION

Head
MIWA, Tomoki

Common Facility Group

Chief: *MORI, Tomoko*

● ***NIBB Core Research Facilities***
Unit Chief: *KONDO, Maki*
Subunit Chief: *MAKINO, Yumiko*
YAMAGUCHI, Katsushi
NISHIDE, Hiroyo
Technical Staff: *NAKAMURA, Takanori*
TANIGUCHI-SAIDA, Misako
BINO, Takahiro
Technical Assistant: *ICHIKAWA, Chiaki*
ICHIKAWA, Mariko
OKA, Naomi
SHIBATA, Emiko

● ***NIBB Bioresource Center***
Unit Chief: *OHSAWA, Sonoko*
MOROOKA, Naoki
Technical Staff: *NOGUCHI, Yuji*
Technical Assistant: *TAKAGI, Yukari*
SUGINAGA, Tomomi
KOTANI, Keiko

● ***Disposal of Waste Matter Facility***
Unit Chief: *MATSUDA, Yoshimi*

● ***Center for Radioisotope Facilities***
Unit Chief: *MATSUDA, Yoshimi*
Subunit Chief: *SAWADA, Kaoru*
Technical Staff: *IINUMA, Hideko*

Research Support Group

Chief: *MIZUTANI, Takeshi*

● ***Cell Biology***
Subunit Chief: *HAYASHI, Kohji*

● ***Developmental Biology***
Subunit Chief: *TAKAGI, Chiyo*
UTSUMI, Hideko
OKA, Sanae
Technical Staff: *MIZUGUCHI, Hiroko*

● ***Neurobiology***
Subunit Chief: *TAKEUCHI, Yasushi*

● ***Evolutionary Biology and Biodiversity***
Unit Chief: *FUKADA-TANAKA, Sachiko*
KABEYA, Yukiko

● ***Environmental Biology***
Technical Staff: *NODA, Chiyo*

Reception

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

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

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

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

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

The 65th NIBB Conference
"Renaissance of *Marchantia polymorpha* – the genome and beyond"

Organizers: John L. Bowman (Monash University, Australia),Takayuki Kohchi (Kyoto University, Japan),Takashi Ueda (National Institute for Basic Biology, Japan)

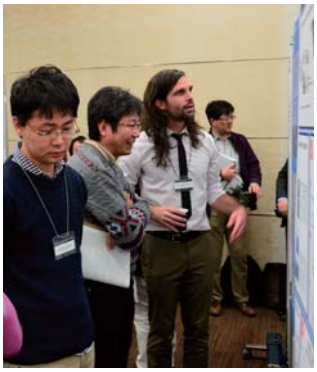
December 16 (Sat) – 18 (Mon), 2017

The 65th NIBB Conference "Renaissance of *Marchantia polymorpha* ~ the genome and beyond" was held at the Okazaki Conference Center from 16th December to 18th December 2017. Professor Takayuki Kohchi (Kyoto University, Japan), Professor John L. Bowman (Monash University, Australia) and I organized the conference. The liverwort "Zenigoke" (*Marchantia polymorpha*) was already described in the literature of pharmaceutical sciences in ancient Greece, and in the past 200 years *Marchantia* has been used as a model plant in modern biology. In the latter half of the 20th century its position as a major model plant was temporarily taken over by other plant species such as *Arabidopsis thaliana*, but in recent years *Marchantia* has been reevaluated because of its merits in the life cycle and body plan as well as its manageability as a research material. Combined with the establishment of various tools and techniques for molecular genetic studies, this plant is now getting hot attention from plant scientists. Furthermore, the paper on the draft genome of *M. polymorpha* was published in the October 5th issue of *Cell*, 2017. Under these circumstances, the NIBB Conference was planned to discuss deeply fundamental biological functions and physiologies of plants as well as to promote future collaborative research.

The conference was held with 30 invited speakers from 10 countries and 115 general participants from 9 countries. Three plenary lectures, 27 invited talks, 16 short talks selected from posters, 6 topics in a technology workshop, and 58 posters were presented. Throughout the entire conference program, including scientific sessions and the banquet on the last day, all participants enjoyed deep discussions from various perspectives, ranging from individual biological phenomena, to the future roles to be played by *Marchantia*, and the future of plant science. After the meeting, we received a lot of messages from both foreign and domestic participants, many of which said "the conference was very meaningful and stimulating for future research". It is a great pleasure for the organizers to have such a fruitful international conference as the NIBB conference.

The organizers appreciate all the participants for their exciting talks and lively discussion. We are also thankful to the Grant-in-Aid for Scientific Research on Innovative Areas (MEXT) "Multidimensional Exploration of Logics of Plant Development", the Interuniversity Bio-Backup Project for Basic Biology (IBBP), the Support Program for Interaction-based Initiative Team Studies (SPIRITS) by Kyoto University, and the Daiko Foundation for financial support. We are also most grateful to the Office of International Cooperation, NIBB for the intensive support. I would like to conclude this message with words from John Bowman, "after the Renaissance at NIBB/OCC… the Enlightenment!".

Takashi Ueda (On behalf of the Organizers)



Speakers

Arteaga-Vazquez, Mario A. (Universidad Veracruzana), Berger, Frederic (Gregor Mendel Institute), Bowman, John L. (Monash Univ.), Chen, Feng (Univ. Tennessee), Dolan, Liam (Univ. Oxford), Grossniklaus, Ueli (Univ. Zurich), Harrison, Jill (Univ. Bristol), Lin, Shih-Shun (National Taiwan Univ.), Nakagami, Hirofumi (MPIPZ), Solano, Roberto (CNB-CSIC), Weijers, Dolf (Wageningen Univ. Research),Aki, Shiori S. (NAIST), Araki, Takashi (Kyoto Univ.), Demura, Taku (NAIST), Hasebe, Mitsuyasu (NIBB), Ishizaki, Kimitsune (Kobe Univ.), Kodama, Yutaka (Utsunomiya Univ.), Kohchi, Takayuki (Kyoto Univ.), Kuchitsu, Kazuyuki (Tokyo Univ. Science), Nakamura, Yasukazu (NIG), Naramoto, Satoshi (Tohoku Univ.), Nishihama, Ryuichi (Kyoto Univ.), Nishitani, Kazuhiko (Tohoku Univ.), Ohta, Hiroyuki (Tokyo Tech.), Sakayama, Hidetoshi (Kobe Univ.), Sakakibara, Keiko (Rikkyo Univ.), Sekimoto, Hiroyuki (Japan Women's Univ.), Shimamura, Masaki (Hiroshima Univ.), Ueda, Takashi (NIBB), Yamato, Katsuyuki T. (Kindai Univ.)

The 19th EMBL International Ph.D. Symposium "Bridging the Gaps – Interdisciplinary Approaches in Life Sciences –"

October 19 (Thu) – 21 (Sat), 2017 | EMBL, Heidelberg

Five PhD students from SOKENDAI/NIBB were funded by NIBB to participate in the 19th EMBL International Ph.D. Symposium "Bridging the Gaps – Interdisciplinary Approaches in Life Sciences –" (held on 19-21 October). Our students had the chance to give poster presentations at the symposium to introduce their current research. They also attended EMBL's laboratories to exchange experimental information and discuss their research with PhD students, post-docs and PIs.

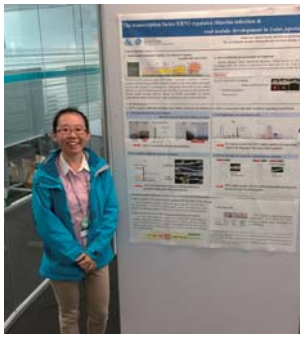
Comments from Students (excerpts)

• Liu Meng

I got some nice advice from my host lab and had an impressive experience in the following symposium.

My host PI, Dr. Rainer Pepperkok and his team members introduced their research to me, and discussed my current studies as well, during the one-day-stay in their lab. My research is generally genetic analysis while Dr. Pepperkok's team is good at cell biology, specifically on ER vesicle traffic. They helped me in making a clear picture of what I can possibly do for the next step and also gave me some detailed advice like what kind of marker might be suitable for me; they literally guided me to build a bridge between my genetic studies and further cellular level analysis. The discussion on their seminar impressed me so much. They are very open to giving and accepting advice from others. At some points the discussion turned heated and they were never afraid of speaking their minds. This was my first time joining a western style discussion and it was really like a brainstorm for me.

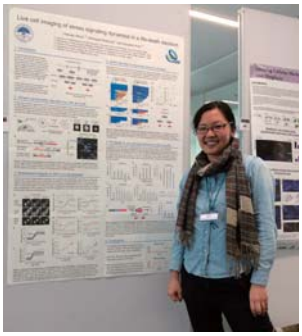
For the next three days we attended the PhD symposium with other EMBL students. Besides all those fantastic eye-opening lectures and talks, I want to talk about our exciting experience at the party on the last day of conference. In Asia we never had this type of party. Seeing all other western students singing and dancing, we debated for a while of whether we would join them or not. Eventually we jumped into the crowd when we realized just standing there was worse than dancing in front of a bunch of people and we fit in right away. We kept drinking and dancing with other EMBL students, enjoying the rare opportunity for relaxing.



Though it was a small step for me, it was an interesting starter to attempt to integrate into western culture.

The four-day trip was short but its effect on me was significant. Besides the useful advice and tips I got from the lab tour and conference, I love my research more than ever before. I'm more comfortable to learn new things in my studies and am not caring so much about loneliness anymore. I hope this trip is a good starter for my two years deferment study here.

• Haruko Miura



The symposium was entirely organized by 2nd year EMBL PhD students, who did a really great job in compiling a fantastic meeting. About 100 PhD students from all over the world and 15 invited speakers participated in the symposium. Many students came from Finland and Australia, since EMBL has partnerships and sites in those countries as well. Surprisingly, there were only a few German students, which might reflect the very international character of EMBL. This year's theme was "Bridging the Gaps - Interdisciplinary Approaches in Life Sciences", so speakers from interdisciplinary fields gave lectures, e.g. synthetic biology, systems biology, tissue engineering, microfluidic technology, and state-of-art fluorescence imaging. Furthermore, there were interesting 'Science and Society' talks and a plenary discussion on topics like talking to journalists, women in science, and career paths. I had the chance to present my research in a poster presentation and short talk to a broad audience and got positive feedback from many fellow students. Inspired by the lectures, I want to apply 3D tissue culture, microfluidics, and new fluorescent tools in my future research. The meeting was not only a good opportunity to network with peers from various countries, but also to connect with the other NIBB students.

On the last day, I visited the Schultz group, who work on the development of chemical tools for live cell imaging and manipulating cellular signaling. Since our fields of study were related, we had lively scientific discussions and they gave me valuable advice for my research. All in all, I had the impression that EMBL is a great place for postdoc studies, due to the cutting edge research, excellent core-facilities with superb technical support, and high internationality. Finally, I want to thank all the involved EMBL and NIBB members for making these valuable experiences possible which will definitely help me in my future research and career.

NIBB-Princeton Joint Proteomics Training Course "– Protein Identification, Quantification and Characterization –"

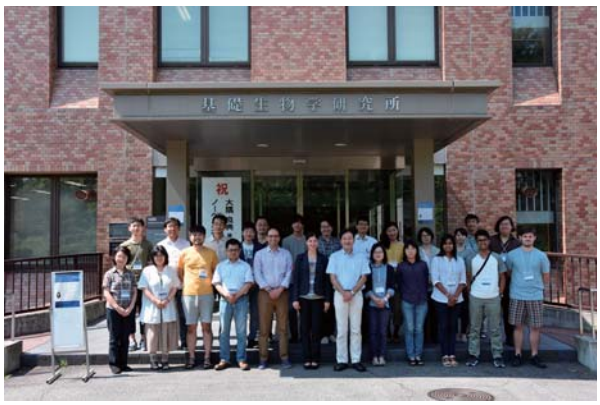
- Period: July 19 (Wed) – 21 (Fri), 2017
- Participants: 17 (including 4 from NIBB)
- Organizer:
  Dr. Naoto Ueno (NIBB), Dr. Shuji Shigenobu (NIBB)
  Dr. Ileana Cristea (Princeton Univ.)
- Lecturers:
  Dr. Ileana Cristea (Princeton Univ.)
  Dr. Todd Greco (Princeton Univ.)
- Program:
  Lecture 1: Introduction to Mass Spectrometry and Proteomics
  Lecture 2: Protein quantification
  Lecture 3: Protein interactions
  Lecture 4: Protein posttranslational modifications
  Tutorial 1: Manual interpretation on MS/MS Spectra
  Tutorial 2: Quantification: introduction to software
  Tutorial 3: Hands-on data analysis
  Hands-on 1: SDS-PAGE staining and imaging, Gel band excision and dehydration, In-gel digestion
  Hands-on 2: Peptide extraction, LC/MS/MS analysis, Demonstration of Mass Spectrometer

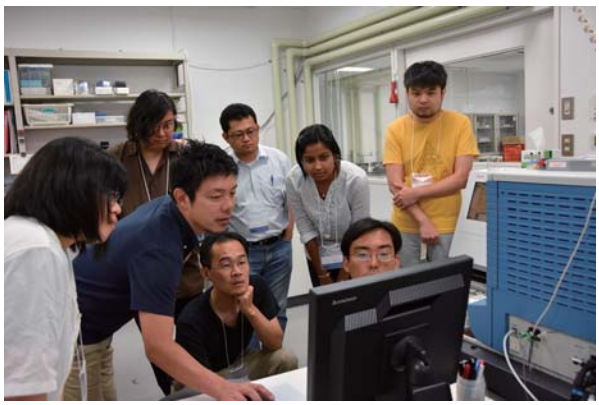
As part of international collaboration and cooperation with Princeton University, we held a proteomics training course "NIBB - Princeton Joint Proteomics Training Course 2017 - Protein identification, quantification and characterization -" from July 19 to 21, 2017. Over the three-day course, we conducted lectures and hands-on training to master principles and measurement techniques on protein identification and quantification by mass spectrometry. From Princeton University, Professor Ileana Cristea, who is a head instructor of the summer Proteomics Course at the Cold Spring Harbor Laboratory and a prominent virus researcher using proteomic approaches, was invited as one of the organizers. Dr. Todd Greco, who is a researcher at Cristea's Laboratory, participated in the course as a lecturer. Participants were selected based on their applications. From 26 domestic and overseas applicants, 17 people, including four NIBB members, participated in the course. There was a wide range of participants including graduate students, postdoctoral researchers, and faculty members and staff of various universities and institutes. Three of them were graduate students and postdoctoral researchers belonging to universities in Japan from foreign countries.

Professor Cristea was mainly responsible for lectures on proteomics from theoretical background to applied research. The hands-on training was supported by the NIBB faculty and staff, and the participants themselves prepared and analyzed protein samples extracted from *Xenopus* embryos. The lectures and hands-on training for bioinformatics of proteomics analysis was handled by Dr. Greco, and the participants conducted data analysis exercises based on the results obtained from the hands-on training using a computer deployed for each group. Due to being divided among four groups, participants deeply interacted with their partners, and lectures and hands-on training were held with active discussion.

Through the preparation and conduction of the course, I believe that the connection between Princeton University and NIBB researchers has deepened. It was also a good opportunity for us to learn the cutting edge analytical techniques used by Princeton University. We gained a lot of hints that I believe will be useful for Collaborative Research Projects for Integrative Genomics utilizing mass spectrometers.

Shuji Shigenobu (On behalf of the Organizer)





The 11th NIBB Bioimaging Forum "Pioneering New Bioimaging by Fusing Optics and Biology"

Organizers: Yasuhiro Kamei, Hideki Takami, Yutaka Hayano, Mitsuo Takeda, Hirotsugu Yamamoto, Masayuki Hattori, Takashi Murata, Shigenori Nonaka, Yosuke Tamada

February 14 (Tue) -15 (Wed), 2017

We held the 11th NIBB Bioimaging Forum on February 14th and 15th 2017. The subtitle for this meeting was "Pioneering New Bioimaging by Fusing Optics and Biology", and aimed at the development of new fields in bioimaging by inviting experts in various disciplines including optical engineering and astronomy to give lectures. Fifty four participants (8 were from NIBB, 46 from outside in which 24 were from private companies) from a wide variety of specialties, such as optics, engineering, mathematics, astronomy, biology, medicine, and agricultural science. The first session of the forum, therefore, included descriptions of characteristics of each field by specialists in biology, optical engineering, and astronomical optics to enhance understanding each other. The succeeding 4 sessions included 15 lectures (30 minutes each with 5 minutes discussion) and 10 posters. Dr. Mitsuo Takeda, a famous theoretical optics specialist, summarized at the end of the forum how we came to understand the "philosophy and condition" of each field, which is necessary to fuse different fields. Forums aiming to establish new methods of bioimaging by fusing different fields are still rare and we believe our forum is functioning well as a start point.

(Yasuhiro Kamei)

The NIBB Genome Informatics Training ourse

The NIBB Core Research Facilities organizes a series of training courses on up-to-date research techniques. The NIBB Genome Informatics Training Course (GITC) is specially designed for biologists who are not familiar with bioinformatics. In 2017, we held two sets of training courses on RNA-seq analysis, a UNIX learning course and a course on BLAST analysis. We provided the RNA-seq analysis course divided into two 2-day programs: one was a preparatory course to learn basics of UNIX and R and the other was a practical course to learn pipelines of RNA-seq analysis using next-generation sequence data. The UNIX learning course was a 2-day program for beginners who are starting bioinformatics. The BLAST analysis course was designed for advanced users aiming to enable participants to get familiar with a large scale sequence database search in the local environment using BLAST software in a robust and systematic manner. These GITC courses offered lectures and hands-on tutorials.

Introduction to RNA-seq – from the basics of NGS to *de novo* analyses

- Organizers: Dr. Shuji Shigenobu and Dr. Ikuo Uchiyama (NIBB Core Research Facilities)
- Lecturers: Dr. Shuji Shigenobu, Dr. Ikuo Uchiyama, Dr. Masanao Sato (Hokkaido Univ.), Mr. Tomoki Miwa, Dr. Katsushi Yamaguchi, Ms. Hiroyo Nishide, Mr. Takanori Nakamura (NIBB Core Research Facilities)

February 23(Thu)-24(Fri), 2017

(Preparatory Course) Basics of UNIX, R, and NGS

- Participants: 23 (including 4 from NIBB)
- Program:
  1. UNIX for beginners
  2. Introduction to "R"
  3. NGS basic data formats and NGS basic tools
  4. Text processing
  5. Shell scripting
  6. Exercises

March 9(Thu)-10(Fri), 2017

(Practical Course) RNA-seq analysis pipeline

- Participants: 21 (including 3 from NIBB)
- Program:
  1. Introduction to statistics
  2. RNA-seq for beginners: genome-based and transcriptome-based approaches
  3. Multivariate statistics
  4. Exercises

Introduction to UNIX for analyses of large scale biological data sets

June 22(Thu)-23(Fri), 2017

- Organizer: Dr. Ikuo Uchiyama (NIBB Core Research Facilities)
- Lecturers: Dr. Ikuo Uchiyama, Mr. Tomoki Miwa (Head of Technical Division), Ms. Hiroyo Nishide, Mr. Takanori Nakamura (NIBB Core Research Facilities)
- Participants: 22 (including 6 from NIBB)
- Program:
  1. UNIX for beginners
  2. Introduction to NGS data analysis
  3. Text processing
  4. Shell scripts
  5. Usage of job management system
  6. Exercises

Mastering BLAST, the essence of sequence analyses

September 14(Thu)-15(Fri), 2017

- Organizers & Lecturers: Dr. Shuji Shigenobu, Dr. Ikuo Uchiyama (NIBB Core Research Facilities)
- Participants: 21 (including 5 from NIBB)
- Program:
  1. BLAST for beginners
  2. Local BLAST search with command-line
  3. BLAST inside – theoretical background of sequence search
  4. Large scale BLAST search
  5. Annotation of genes and ortholog analysis
  6. Beyond BLAST

The 5th BioImage Analysis Training Course

Organizers: Dr. Kagayaki Kato, Dr. Yoshitaka Kimori, Dr. Yasuhiro Kamei, Dr. Hiroshi Koyama, Dr. Shigenori Nonaka, Dr. Takashi Murata.
Supervisors: Prof. Naoto Ueno, Prof. Toshihiko Fujimori.

November 20 (Mon) -22 (Wed), 2017

The 5th BioImage Analysis Training Course was held jointly by the Center for Novel Science Initiatives' Department of Imaging Science, JSPS KAKENHI Platforms for Advanced Bioimaging Support (ABiS), and NIBB. This course was designed for biologists who are relatively new to analyzing datum obtained through advanced microscopy. Therefore the focus of training was learning image processing and analytical techniques through "solving simple problems with image analysis" and "understanding appropriate methods and necessary preparation for consulting experts in technically advanced imaging challenges". 47 people applied for the course, which had a maximum capacity of 18 participants, this clearly suggests the height of the demand for courses on these subjects.

This course's lectures were conducted with the aim of training participants to keep in mind the series of steps essential to fundamental image processing and analysis while obtaining images to be used (workflows). In addition, we loaned the participants PCs pre-installed with ImageJ, a typical open-source software package for biological image processing and analysis, and images which were used for practicing the basic operations and settings of image processing. Also, lectures were given on how programming of simple "macro-programs" which use these workflows in ImageJ allows automation, which is essential for the large capacity and high-dimensional throughput of microscopic imaging which has become common in recent years.

At the conclusion of the course each of the students gave commentary and discussed the methods used with examples of actual images from their own research. Every year after the course, participants express feeling "pretty tired, and satisfied" as part of the questionnaire, and certainly there is a true benefit in terms of their image analysis by becoming more familiar with these techniques. In addition, we expect that this course will increase opportunities for joint research relating to biological image analysis.

(Kagayaki Kato)

Advanced Bioimaging Support (ABiS)

In the field of life science, the demand for bioimaging has increased in recent years. However, due to advances in imaging technologies, i.e., the diversification and specialization of imaging equipment and increasingly complex operations, increased costs of equipment and running costs, and the growing need for image data analysis, individual research institutes and universities have encountered increasing difficulties related to the introduction, maintenance, and operation of imaging equipment.

ABiS was launched as one of the "Platforms for Advanced Technologies and Research Resources" in fiscal year (FY) 2016 under the new framework of the Grant-in-Aid for Scientific Research on Innovative Areas (Leader: Prof. Masanobu KANO, NIPS/The University of Tokyo). This program aims to contribute the further development of academic research in Japan through providing cutting-edge equipment and methodologies to individual KAKENHI (Grants-in-Aid for Scientific Research) research by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) under the Grant-in-Aid for Scientific Research on Innovative Areas (FY2016-FY2021).

ABiS provides cutting-edge instruments for light microscopy, electron microscopy, magnetic resonance imaging, and other methods through the network in cooperation with domestic partner organizations that own and operate multiple types of advanced specialized imaging equipment, in order to provide comprehensive support for advanced imaging in the field of life science. NIBB together with NIPS contribute as core institutes to the ABiS network. Of the various ABiS support activities, NIBB is in charge of the following:

- Light microscopy
  - 4D microscopy by Prof. Toshihiko FUJIMOIRI
  - IR-LEGO microcopy by Assoc. Prof. Yasuhiro KAMEI
  - DSLM by Assoc. Prof. Shigenori NONAKA
- Imaging analysis
  - Development of image processing/analysis algorithms for biological data by Prof. Naoto UENO and Assist. Prof. Kagayaki KATO
- Training
  - Training for image analysis by Assist. Profs. Yoshitaka KIMORI and Hiroshi KOYAMA

To organize and coordinate ABiS activities, two secretariat offices were established in NIBB (Assoc. Prof. Shoji MANO) and NIPS, respectively, under the control of the general support group (The persons in charge in NIBB; Director-general Prof. Masayuki YAMAMOTO, Prof. Naoto UENO, Prof. Shinji TAKADA, Assoc. Prof. Shoji MANO). General support includes the budget planning and management of ABiS activities. In particular, we promote ABiS activities via the website and other media so that KAKENHI researchers make full use of the ABiS platform to accelerate their research projects. In addition, we organize technology training sessions, workshops, and symposia to disseminate advanced imaging technologies and share information about them. We also coordinate with the other three platforms (Platform of Supporting Cohort Study and Biospecimen Analysis, Platform of Advanced Animal Model Support, and Platform for Advanced Genome Science) to provide multidisciplinary and international support.

The 1st ABiS Symposium "Towards the Future of Advanced Bioimaging for Life Sciences"

February 19 (Sun) ~ 20 (Mon), 2017

Sponsored by: Advanced Bioimaging Support

Co-sponsored by:
National Institutes of Natural Sciences
National Institute for Physiological Sciences
National Institute for Basic Biology
Center for Novel Science Initiatives
Grant-in-Aid for Scientific Research on Innovative Areas-'Resonance Bio'
Grant-in-Aid for Scientific Research on Innovative Areas-'The Birth of New Plant Species'
Grant-in-Aid for Scientific Research on Innovative Areas — Platforms for Advanced Technologies and Research Resources "Committee on Promoting Collaboration in Life Sciences"

Participants: 151

Oral presentation: 18

Poster presentation: 54

The 1st ABiS symposium was held in Okazaki to exchange information on the state-of-the-art technologies for imaging. This symposium was open to researchers who are interested in imaging techniques in addition to researchers involved in the ABiS network. In this symposium, we introduced ABiS activities and the research results of the support. In addition, two research groups of 'Grant-in-Aid for Scientific Research on Innovative Areas' in which bio-imaging is a core methodology, and two foreign programs, Global Bioimaging promoted by Euro-Bioimaging and Center for Bioimaging Sciences in National University of Singapore, introduced their activities related to the promotion of imaging sciences.

The NIBB Internship program

The NIBB Internship program, started in 2009, is a hands-on learning course for overseas students designed to give high-quality experience in real world research and focused education of biology. At the same time, this program aims to internationalize the graduate students of SOKENDAI (Graduate University for Advanced Studies), giving them the opportunity to get to know students and interns with various cultural customs. Another goal of the program is to build connections through providing education to the people who will form the core of international research networks in the future.

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

In FY 2017 there were 57 applicants, out of which seven interns were selected. These interns were from universities located in six countries (Colombia, Germany, Indonesia, Malaysia, Republic of Serbia, and Japan) and spent periods ranging from two to twelve weeks experiencing life as a member of a research team. Moreover, two interns from China stayed at NIBB for twelve weeks and one intern from France stayed one week by their own travel grants.

Report from a participant
Olivera Valentirović
University of Belgrade, Serbia

My name is Olivera Valentirović and I am an undergraduate student at Faculty of Biology at the University of Belgrade, Serbia. Thanks to prof. Dr Nakayama, who suggested the NIBB internship program to me, I was able to experience working at his laboratory for one month. It was a great adventure and opportunity for me, since I had a chance to work in the field of epigenetics and to travel to a breath-taking far away country, that was something I only dreamt about before.

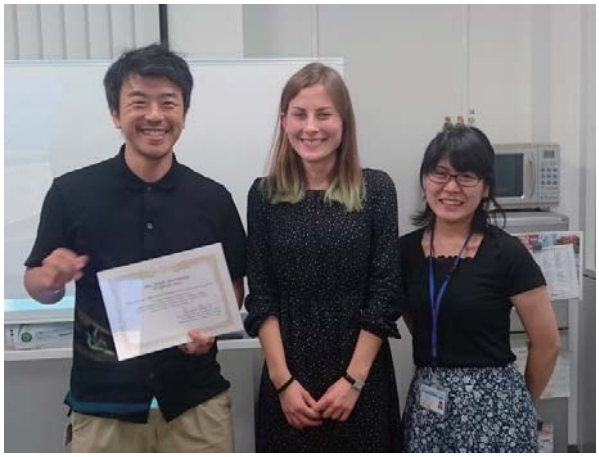
The research I performed during my internship at NIBB was on the ciliate *Tetrahymena thermophila*. This was my first time working in a laboratory as well as with *Tetrahymena*. I found its life cycle and the extreme processes happening on its genome during its sexual reproduction process called conjugation extremely fascinating. Those peculiarities make it a really favorable and attractive model organism.

My project was to characterize the proteins involved in the formation of heterochromatin and DNA elimination during genome rearrangement within the macronucleus. In one experiment I characterized several cell lines that express proteins tagged with green fluorescent protein to analyze localizations of heterochromatin protein candidates. It was a great experience seeing gene gun transformation for the first time. I also learned a lot about culturing, storing and transferring *Tetrahymena* and how to perform a selection of the transformed cells and phenotype assortments using different concentrations of selective substances in the medium. In a parallel experiment, I aimed to reveal the sufficiency of few proteins for DNA elimination by a tethering assay. I faced some difficulties during this experiment, but I found it a good experience for my future work, that I may face and need to solve on a daily basis. Every day I was presented with interesting challenges and new chances to find a way to untangle some problem. In the end, I managed to go through all the intended steps and successfully finish the experiments with some of the samples. I wish I could have had a longer period of time to do more experiments with different conditions, and finish all of the initially planned experiments.

Accommodation at Myodaiji Lodge was extremely comfortable which made my stay very pleasant. I didn't lack anything and everything was professionally organized, thanks to the International Corporation Office.

All of the lab members of the Division of Chromatin Regulation were always willing to help me with my work, as well as with my stay. They gave me a lot of precious advices for which I am immensely grateful. It is a great honor for me to have worked with such polite, kind and hard-working people. I would especially like to thank prof. Dr Nakayama who gave me this opportunity to join his lab and offered me an unforgettable experience of Japanese culture, its delicious cuisine and its people. I am eternally thankful to Dr Kataoka from whom I've learned many valuable things, who patiently and carefully watched and guided me through every step and who showed me how wonderful and incredibly fun working in the laboratory can be. I really appreciate friendly and welcoming atmosphere during my stay.

This was a life-changing adventure for me. In addition to the exciting bench work in the actual lab, I've gained many unforgettable memories, visited amazing places, felt one unique culture, met remarkable people and made lasting friendships. The whole experience inspired me to pursue my career in science even more than before.



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Access

From Tokyo

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

From Osaka

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

From Central Japan International Airport

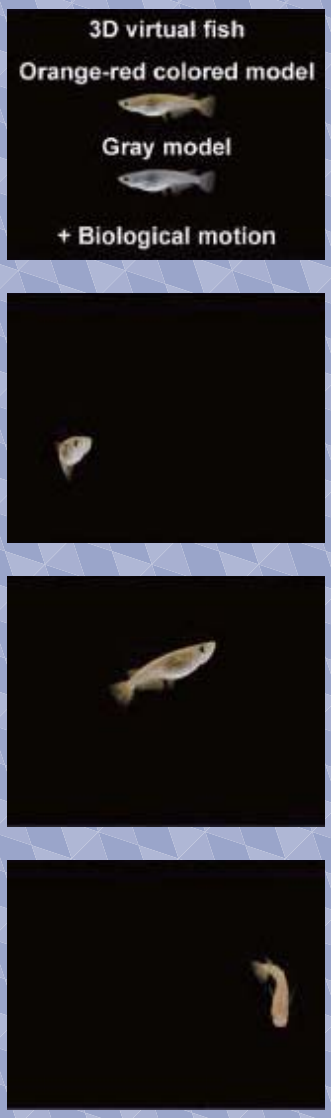
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

From Higashi Okazaki Station to Each Area

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

By car

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



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