National Institute for Basic Biology

2018 ANNUAL REPORT

CONTENTS

The cover items are related to a paper titled "Repeated inversions within a *pannier* intron drive diversification of intraspecific colour patterns of ladybird beetles" (Ando *et al*., Nature Communications 2018) from the laboratory of Prof. Niimi. The paper demonstrated that a single transcription factor gene regulates the highly diverse elytral color patterns in the Asian multicolored ladybird beetle *Harmonia axyridis*. See page 55 of this report for details.

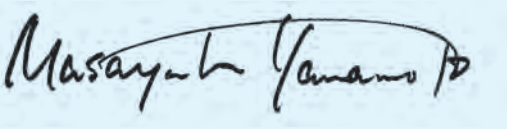
It is my great pleasure to present to you all the 2018 Annual Report of the National Institute for Basic Biology (NIBB), which outlines the Institute's research activities and its effective function as a center for collaborative research in Japan over the last year. Given these important institutional missions, I have tried to maintain the liberal and forward-looking atmosphere for research and discussion that NIBB has sustained for many years, which I believe is an essential basis for these remarkable activities.

As I will finish my term at the end of March 2019, this will be the last Annual Report issued under my directorship. I cordially thank you all for the warm support and encouragement that you have given me during the last six years. I sincerely hope that NIBB will continue to open up and foster new research fields in biology and that every person in NIBB will do his/her best in producing good research, as well as upholding high ethical and compliance standards. Good science, even in a basic research field, will eventually benefit human beings. The history of science tells us this is immutably true. I hope that NIBB will continue to work hard to be truly acknowledged as a remarkable institution.

Please find in this booklet a summary of NIBB's research, collaborative, educational, and international activities in 2018. I would like to note that we welcomed several new colleagues in 2018, including one professor, one specially appointed associate professor, three assistant professors, two specially appointed assistant professors, and four NIBB research fellows, while three colleagues transferred to other institutes.

I would also like to congratulate Professor Masaharu Noda, Dr. Takashi Matsuda, and Dr. Haruka Kawaguchi for winning awards, 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 29, 2019

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 designated to promote and stimulate the study of biology both in Japan and internationally. 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, developmental, evolutionary, environmental and theoretical biology as well as neurobiology.

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.

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 from outside of it, 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.

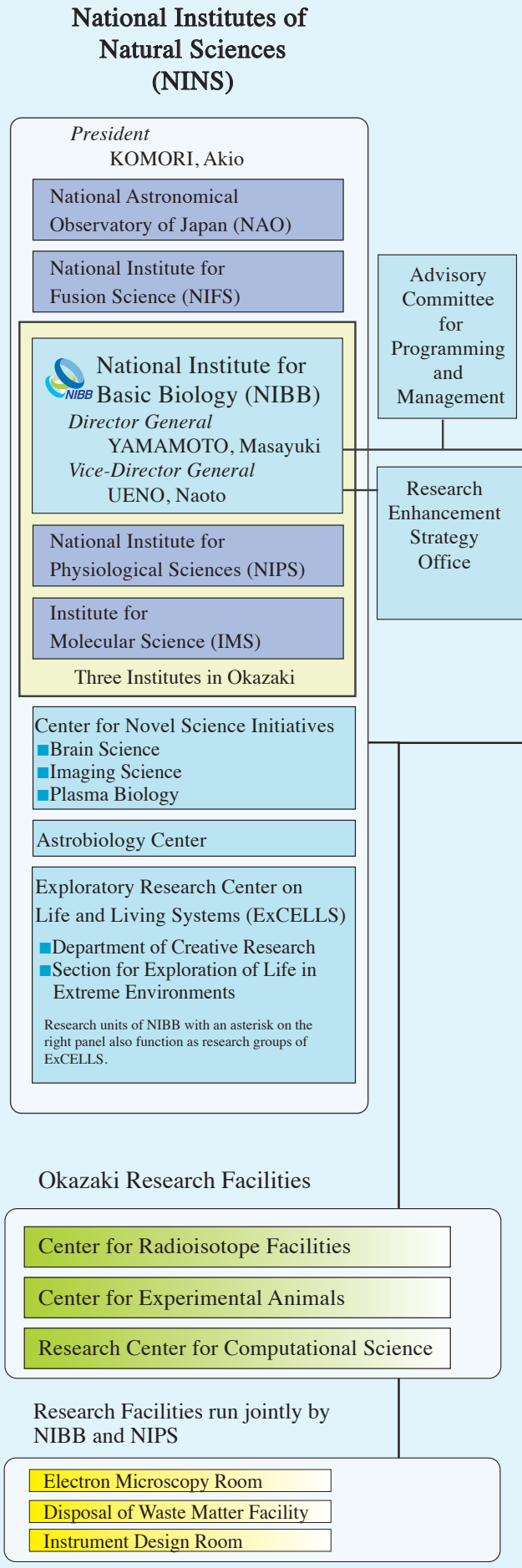
The Research Enhancement Strategy Office, aimed at supporting researchers in order to improve NIBB's abilities as a collaborative research institution, was founded in 2013. The Office is made up of six groups (p. 97) and its activities are mainly carried out by URAs (University Research Administrators) 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.

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

Organization



National Institute for Basic Biology (NIBB)

As of January 31, 2019

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
  - Laboratory of Organelle Regulation
- **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 Plant Environmental Responses
  - Division of Seasonal Biology (Adjunct)
- **Theoretical Biology**
  - Laboratory of Genome Informatics
- **Imaging Science**
  - Laboratory for Spatiotemporal Regulations*

- **Concurrent Faculty**
  - Laboratory of Nuclear Dynamics
  - Laboratory of Plant Development and Physiology
  - NINS Astrobiology Center

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 for the Interuniversity Bio-Backup Project (IBBP Center)**
- **Center for the Development of New Model Organisms**

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

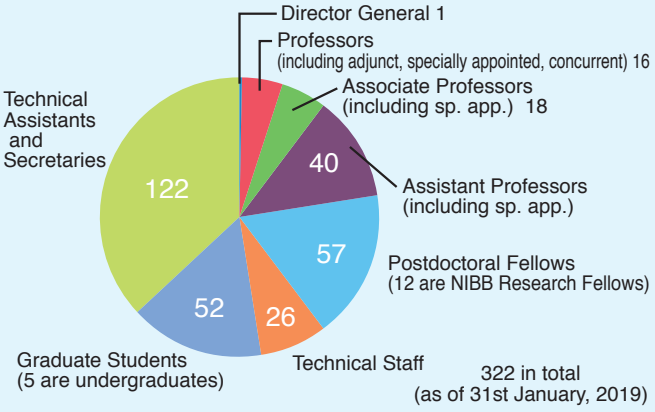
- Evaluation and Information Group
- Public Relations Group
- International Cooperation Group
- Collaborative Research Group
- Young Researcher Support Group
- Gender Equality Promotion Group

- **Okazaki Administration Office**

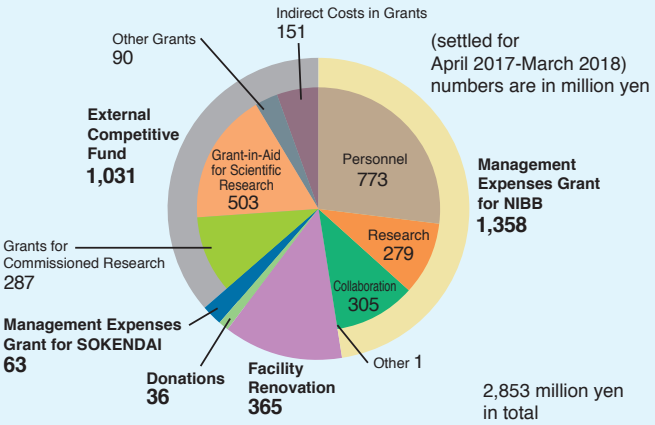
Research Facilities and NIBB BioResource Center were launched in 2010 to support research in NIBB and also to act as an intellectual hub to promote collaboration among the researchers of NIBB and other institutions. The NIBB Center of the Inter-University Bio-Backup Project (IBBP Center) was founded in 2012 to prevent the loss of invaluable biological resources. Projects for the development of bioresource preservation technology are solicited by the IBBP center. The Center for the Development of New Model Organisms 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. The Center for Radioisotope Facilities is one of the latter and run by the technical staff of NIBB.

The Exploratory Research Center on Life and Living Systems (ExCELLS) was founded in 2018 to develop novel approaches for observing biological entities, deciphering hidden information, and creating living systems to improve our understanding of their nature. Moreover, ExCELLS promotes collaborative, interdisciplinary research involving investigators exploring organisms living in extreme environments.

Members in NIBB



Financial Configuration of NIBB



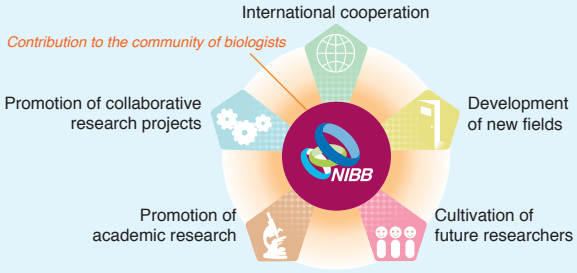
Members of the Advisory Committee for Programming and Management# (as of January, 2019)

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

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 that are conducted using NIBB's facilities and in collaboration with NIBB's divisions/laboratories are solicited from external researchers. "Individual Collaborative Research Projects" are a basic method of supporting collaborations which provide external researchers with travel and lodging expenses to visit NIBB's laboratories to conduct collaborative research. "Priority Collaborative Research Projects" are carried out as group research projects 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. Research expenses in addition to travel expenses are provided for these projects. 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 also provided for these projects.

year	2016	2017	2018
Priority collaborative research projects	2	2	1
Collaborative research projects for model organisms and technology development	2	2	2
Individual collaborative research projects	46	51	57
Collaborative research projects for integrative genomics	59	62	67
Collaborative research projects for integrative bioimaging	38	28	23
NIBB workshops	6	3	2
Collaborative experiments using the Large Spectrograph	10	9	9
Support for NIBB training courses	0	0	1
Collaborative research projects for bioresource preservation technology development	12	12	18
total	175	169	180

NIBB Core Research Facilities

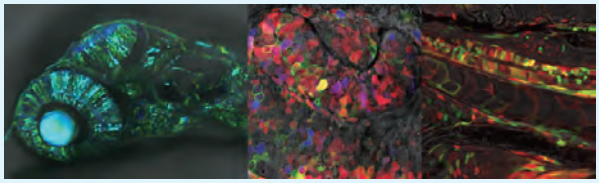
The NIBB Core Research Facilities support research at NIBB and also act as an intellectual hub to promote collaboration between NIBB and other academic institutions. They consist of three facilities that are developing and providing state-of-the-art technologies through functional genomics, bioimaging and bioinformatics (p. 80).

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. Its current focus is supporting functional genomics projects that utilize mass spectrometers and DNA sequencers, and holding events such as training courses to achieve this end (p. 103). 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 task specially appointed associate professors, who are experts in their respective fields, with managing each facility as well as conducting their own academic research. The Data Integration and Analysis Facility supports the analysis of large-scale biological data, such as genomic sequence data, gene expression data, and imaging data. The facility maintains high-performance computers with large-capacity storage systems for this purpose.

NIBB BioResource Center

The NIBB BioResource Center supports research using model animals and plants at 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 glories, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, and provides technical support and advice for the appropriate use of these organisms (p. 87).

The center also acts 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 selected as a center for research on medaka (*Oryzia latipes*), whose usefulness as a vertebrate model was first demonstrated 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 glories and zebrafish (p. 90).



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 for the Interuniversity 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 used in cutting-edge research (p. 91).

Center for the Development of New Model Organisms

This center was established in 2013 and employs a newly assigned cross-appointment researcher who developed and refined genome editing techniques in particular in *Pleurodeles waltl* (Iberian ribbed newt) (p. 94) and also taught these techniques in the NIBB International Practical Course (p. 101).

Advanced Bioimaging Support (ABiS)

ABiS provides assistance for advanced imaging in 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 and specialized imaging equipment (p. 105).

International Cooperation and Outreach

Collaborative Programs with Overseas Institutes

NIBB plays 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. The NIBB-Princeton Joint Proteomics Training Course was held in July, 2017.

NIBB Conference

The NIBB Conferences are international conferences on prominent topics in biology that are 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 66th conference "Cutting Edge Techniques of Bioimaging" will be held jointly with ABiS in February, 2019.

International Practical Course

With the cooperation of researchers from Japan and abroad, the NIBB international practical course is held in a specifically prepared laboratory. The 10th course "Genome Editing and Imaging of Fish and Amphibians" was held jointly with ABiS in September, 2018 at NIBB. Graduate students and young researchers from various areas including the UK, Columbia, Nepal, Korea, China, Taiwan, and Japan, were provided with training in state-of-the-art research techniques (p. 101). International conferences and courses are managed by the International Cooperation Group of the Research Enhancement Strategy Office (p. 98).

Outreach

NIBB's outreach activities aim to present cutting edge research results to the public via mass media through press releases or directly through internet based platforms, such as web pages, Facebook, and Twitter. 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 undergraduate and younger students through lectures and workshops. Outreach activities are mostly managed by the Public Relations Group of the Research Enhancement Strategy Office (p. 97).

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 an electrically tunable lens (p. 76). The Advisory Committee on Bioimaging, comprised of leading researchers in the bioimaging field in Japan, has been organized to formulate advice concerning NIBB's imaging research. The Bioimaging Forum provides an opportunity for researchers and company engineers to frankly discuss practical difficulties and their needs regarding imaging. The 12th Forum "The Future of Bioimage Analysis Explored by AI" was held in March, 2018 (p. 103). A training course in bioimage analysis was also held in 2018 (p. 104).

Okazaki Biology Conferences

NIBB holds Okazaki Biology Conferences (OBC) that 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 that seek strategies for addressing critical future issues in biology. Past conferences have promoted the formation of international researcher communities.

Cultivation of Future Researchers

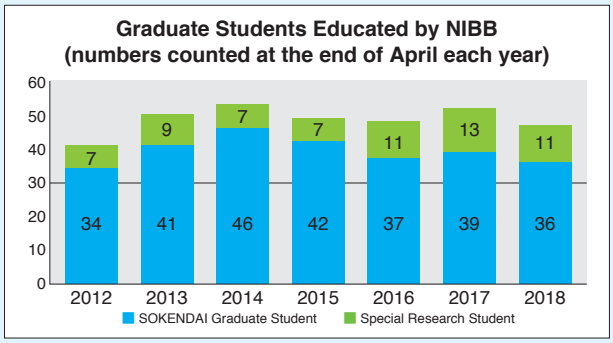
NIBB constitutes the Department of Basic Biology in the School of Life Science of SOKENDAI (Graduate University for Advanced Studies). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree. Graduate students enrolled 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 at least once during their master's and doctoral program, where they are provided with an opportunity to give oral and poster presentations.

Students from Japan and abroad can also come to NIBB through our Internship Program. Internships give students an excellent opportunity to build international connections while experiencing hands on research in a world class research institute (p. 107).

Support for young researchers is managed by the Young Researcher Support Group of the Research Enhancement Strategy Office (p. 99).



Personnel changes in 2018*

Newly assigned to NIBB

Name	Position	Research Unit	Date
OTSUBO, Yoko	Specially Appointed Assistant Professor	Laboratory of Cell Responses (Jointly with NIFS)	February 1
MORITA, Miyo T.	Professor	Division of Plant Environmental Responses	April 1
SUZUKI, Ken-ichi	Specially Appointed Associate Professor	Center for the Development of New Model Organisms (Cross-appointment with Hiroshima Univ.)	April 1
MINAMINO, Naoki	NIBB Research Fellow	Division of Cellular Dynamics	April 1
IKEDA, Tatsuro	NIBB Research Fellow	Division of Germ Cell Biology	April 1
NISHIUMI, Nozomi	NIBB Research Fellow	Laboratory of Neurophysiology	April 1
KIM, Eunchul	NIBB Research Fellow	Division of Environmental Photobiology	April 1
NAKAMURA, Taro	Assistant Professor	Division of Evolutionary Developmental Biology	June 1
NISHIMURA, Takeshi	Assistant Professor	Division of Plant Environmental Responses	September 1
ODA, Shigekazu	Assistant Professor	Division of Quantitative Biology	November 1
OHTA, Yusaku	Specially Appointed Assistant Professor	Laboratoty of Biological Diversity	December 1

Newly affiliated with other universities and institutes

Name	New Affiliation	Position	Date
KIMORI, Yoshitaka	Fukui University of Technology	Associate Professor	April 1
YASUGI, Masaki	Utsunomiya University	Researcher (Industry-Government-Academia Collaboration)	April 1
AIHARA, Yusuke	Nagoya University	Researcher	April 1

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

Awardees in 2018

Name	Position	Award
NODA, Masaharu	Professor	The 2nd Seitai-no-Kagaku (Science of the Living Body) Award
MATSUDA, Takashi	NIBB Research Fellow	The 3rd Toshihiko Tokizane Memorial Award for Excellent Graduate Study in Neuroscience
KAWAGUCHI, Haruka	Postdoctoral Fellow	The 21st ESPEC Prize for the Encouragement of Environmental Studies

Selected Press Releases from NIBB in 2018

9 January

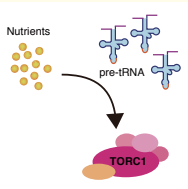
The origin of flower making genes

Flowers are reproductive organs, which are formed by a group of genes called MADS-box genes. Although non-flowering plants are also known to have these genes, it was previously unclear how they worked. Consequently, a research team at NIBB using the moss *Physcomitrella patens* revealed that MADS-box genes control sperm motility, cell division, and elongation of gametophore stems. This finding indicates that MADS-box genes extensively changed their functions during the evolution of land plants (see p. 49).

Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T. and Hasebe, M. (2018). Physcomitrella MADS-box genes regulate water supply and sperm movement for fertilization. Nat. Plants 4, 36-45. doi: 10.1038/s41477-017-0082-9

18 January

tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway

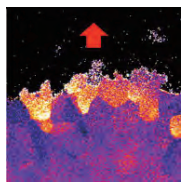


Cells control growth and differentiation in response to their changing external environments. TOR complex 1 (TORC1) is a kinase complex conserved throughout eukaryotes and plays a key role in this control system. In the fission yeast *Schizosaccharomyces pombe,* TORC1 has been known to control the switch between asexual and sexual growth, but the mechanism of how TORC1 recognizes nutrient conditions has remained mostly unknown. NIBB researchers revealed that the precursors of tRNA, which is one of the types of classical non-coding RNA that are indispensable in protein synthesis, operate in the *S. pombe* TORC1 pathway to switch a cell's growth mode from vegetative to reproductive in response to the nutrient conditions (see p. 23).

Otsubo, Y., Matsuo, T., Nishimura, A., Yamamoto, M., and Yamashita, A. (2018). tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway. EMBO Rep. *19*, e44867. doi: 10.15252/embr.201744867

7 February

Controlling cell movement during gastrulation: elevation of intracellular calcium at the leading edge is critical for cell movement

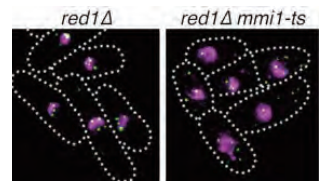


Gastrulation is a large-scale cell movement in the process of animal body formation, and is quite important in the positioning of various body structures. NIBB researchers succeeded in observing changes in intracellular calcium concentrations in moving cell groups during gastrulation in a *Xenopus* embryo. They subsequently found that calcium oscillates only at the leading edge of the cell group, and revealed that this localized elevation of intracellular calcium controls cellular movement during gastrulation (see p. 30).

Hayashi, K., Yamamoto, T.S., and Ueno, N. (2018). Intracellular calcium signal at the leading edge regulates mesodermal sheet migration during *Xenopus* gastrulation. Sci. Rep. *8*, 2433. doi: 10.1038/s41598-018-20747-w

13 February

A novel control mechanism of the expression of genes related to germ cell formation: the transcripts of genes promoting meiosis are tethered to nuclear foci and sequestered from the translation machinery during somatic mitoses

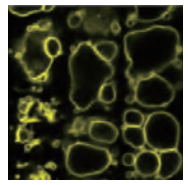


During the formation process of germ cells such as sperm and eggs, a set of genes that differ from somatic cells are expressed. The genes controlling meiosis, which is a form of cell division essential in the formation of germ cells, are known to be regulated not only at the transcriptional level but also at the stability of the transcripts. NIBB researchers revealed that the transcripts of genes promoting meiosis in fission yeast are transcribed at a low level during the mitotic cell cycle but degraded selectively. Even when selective RNA degradation is impaired, these transcripts are tethered to focal structures in the nucleus and inhibited from being translated into protein products (see p. 23).

Shichino, Y., Otsubo, Y., Kimori, Y., Yamamoto, M., and Yamashita, A. (2018). YTH-RNA-binding protein prevents deleterious expression of meiotic proteins by tethering their mRNAs to nuclear foci. eLife *7*, e32155. doi: 10.7554/eLife.32155

■ 20 February

Discovery of the mechanism of plant-specific control of vacuole transport routes

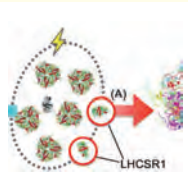


Plant vacuoles have a variety of functions. As such, they have diverse vacuolar transport pathways. An NIBB research team demonstrated that a plant-specific vacuole transport pathway is controlled by CORVET: one of the tethering factors acting in the transport pathway to the vacuole. They also showed that the combination of a factor from a common ancestor and a factor newly acquired by plants has resulted in the diversification of the process of transportation routes to vacuoles. In particular, the group initially examined the relationship between RAB GTPase, CORVET and HOPS using *Arabidopsis thaliana*. It was found that RAB 5 acts on CORVET and endosomes, and that RAB 7 works on HOPS and vacuolar membranes (see p. 14).

Takemoto, K., Ebine, K., Askani, JC., Krüger, F., Ito, E., Goh, T., Schumacher, K., Nakano, A., and Ueda, T. (2018). Distinct sets of tethering complexes, SNARE complexes, and Rab GTPases mediate membrane fusion at the vacuole in Arabidopsis. Proc. Natl. Acad. Sci. USA *115*, E2457-E2466. doi: 10.1073/pnas.1717839115

■ 20 March

Discovery of a novel mechanism that allows plants to release excess light energy

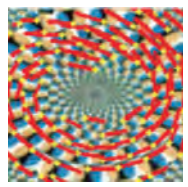


Plants utilize light energy during the process of photosynthesis to convert carbon dioxide and synthesize sugar molecules. An environment with strong light therefore may seem to be preferable for plants, but in many cases the solar light on the earth's surface exceeds the endurable limit of the photosynthetic apparatus. This means that direct exposure to sunlight may have a deleterious effect on plants. Plants have evolved to have mechanisms that intentionally release energy from excessively strong light. As such, NIBB researchers have endeavored to elucidate one of these mechanisms, qE quenching, at the molecular level. They utilized the green alga *Chlamydomonas reinhardtii* and found a novel mechanism that releases the excess energy to Photosystem I through a protein called LHCSR1 (see p. 68).

Kosuge, K., Tokutsu, R., Kim, E., Akimoto, S., Yokono, M., Ueno, Y., and Minagawa, J. (2018). LHCSR1-dependent fluorescence quenching is mediated by excitation energy transfer from LHCII to photosystem I in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *115*, 3722-3727, doi: 10.1073/pnas.1720574115

■ 20 March

Illusory motion reproduced by deep neural networks trained for prediction



Deep neural networks (DNNs) have achieved notable success in a broad range of fields in which they have produced results comparable to the performance of human experts. In line with this, a research team at NIBB successfully reproduced illusory motions by DNNs trained for prediction. This research supported the idea that the mechanism assumed by the predictive coding theory is a basis of motion illusion generation. The DNNs were trained with videos of motion from the point of view of someone watching them, and the motion prediction ability of the obtained computer model was verified using a rotating propeller in unlearned videos. The computer model accurately predicted the magnitude and direction of motion of said rotating propeller in these videos (see p. 48).

Watanabe, E., Kitaoka, A., Sakamoto, K., Yasugi, M. and Tanaka, K. (2018). Illusory motion reproduced by deep neural networks trained for prediction. Front. Psychol. *9*, 345. doi: 10.3389/fpsyg.2018.00345

■ 4 April

Predicting the presence of a new diffusible factor during the formation of a regular pattern of phyllotaxis: a simulation utilizing a mutual interaction model 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. This regular pattern has been reported to be formed by the mutual interaction between a plant hormone, auxin, and its membrane transporter protein PIN1, but its regulation mechanisms remain mostly unknown. NIBB researchers revealed through a computer simulation that a realistic model introducing the extracellular space between neighboring cells does not produce a regular pattern only with auxin and PIN1, but that by assuming the presence of a hypothetical diffusible factor, the regular pattern is reproduced. They also predicted that auxin does not directly regulate PIN1, but needs to indirectly regulate it through a diffusible factor (see p. 52).

Fujita, H., and Kawaguchi, M. (2018). Spatial regularity control of phyllotaxis pattern generated by the mutual interaction between auxin and PIN1. PLoS Comput. Biol. *14*, e1006065. doi: 10.1371/journal.pcbi.1006065

5 April

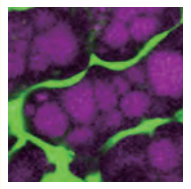
Non-invasive measurement of the tissue stiffness with live-cell time-lapse imaging

When a fertilized egg transforms into an animal's body during development, cell populations forming tissues can be easily deformed. Cells also generate mechanical forces during development, shaping the tissues and organs. In order to understand the process of tissue and organ formations, it is of particular importance to understand how the physical characteristics of tissues responding to mechanical stresses influence the process of organogenesis. To tackle this problem, a joint National Institute for Basic Biology (NIBB)/Kyoto University research team has proposed a unique non-invasive way of measuring the stiffness of tissues by combining physical modeling of the tissues with statistical estimation. The research team developed methodology for estimating tissue stiffness based on the tissue deformation and the mechanical forces applied over the tissue (see p. 17).

Kondo, Y., Aoki, K., and Ishii, S. (2018). Inverse tissue mechanics of cell monolayer expansion. PLoS Comput Biol. *14*, e1006029. doi: 10.1371/journal.pcbi.1006029

15 May

The generality and diversity of life explored through the transportation mechanisms in plant cells: a discovery of a new regulatory mechanism in plant membrane traffic



All life forms are composed of cells and their activities are mostly similar throughout different species. However, the set of component proteins are different and the mechanisms that distribute proteins within a cell also differ between species. NIBB researchers have studied the mechanism of transporting proteins to the plant-specific organelle, the vacuole, and found that plant-specific proteins called ARA6 and PUF2 suppress or activate RAB5, which is also present in animal and fungi. It then accurately controls transportation to the vacuole. The plant devised a unique controlling factor and has succeeded in providing diversity to cellular activity also present in other life forms (see p. 14).

Ito, E., Ebine, K., Choi, S.-w., Ichinose, S., Uemura, T., Nakano, A., and Ueda, T. (2018). Integration of two RAB5 groups during endosomal transport in plants. eLife 7, e34064. doi: 10.7554/eLife.34064

10 July

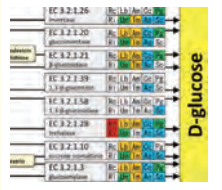
Greatly improved decoding of the genome of Arbuscular mycorrhizal fungus (AMF) led to the identification of nutrients that AMF receives from plants and the discovery of a unique gene structure

AMFs penetrate their hyphae into the soil and also into the roots of plants, which enables them to deliver nutrients deep within the soil to the plants that live in it, and receive photosynthesis products from plants. NIBB researchers decoded the genome of a representative AMF, *Rhizophagus irregularis*, at a considerably higher resolution and confirmed that AMFs lack enzyme genes necessary to synthesize nutrients such as fatty acids or vitamin B1. This information on nutrient requirements may lead to a large-scale culture of AMFs. They also found a unique gene structure in the ribosome DNA gene (see p. 52).

Maeda, T., Kobayashi, Y., Kameoka, H., Okuma, N., Takeda, N., Yamaguchi, K., Bino, T., Shigenobu, S., and Kawaguchi, M. (2018). Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*. Commun. Biol. *1*, 87. doi: 10.1038/s42003-018-0094-7

11 July

Comparative genome analysis of Arbuscular mycorrhizal fungi (AMFs) revealed a common genetic basis for auxotrophy among AMFs



AMFs are representative symbionts with land plants and require nutrients from plants to grow (auxotrophy). To analyze this requirement, NIBB researchers decoded the sequence of an AMF, *Rhizophagus clarus*, and compared it with the genome of a representative AMF, *R. irregularis*. These two AMFs lack the enzymes necessary to synthesize fatty acids or thiamine, and they also lack the enzymes required to degrade polysaccharides into glucose. This indicates that AMFs depend on the supply of fatty acids, thiamine, and glucose from host plants and also suggests the possibility of culturing AMFs, which in turn will enable detailed analyses of the mechanisms of AMF's growth and may lead to agricultural applications (see p. 52).

Kobayashi, Y., Maeda, T., Yamaguchi, K., Kameoka, H., Tanaka, S., Ezawa, T., Shigenobu, S., and Kawaguchi, M. (2018). The genome of *Rhizophagus clarus* HR1 reveals a common genetic basis for auxotrophy among arbuscular mycorrhizal fungi. BMC Genomics *19*, 465. doi: 10.1186/s12864-018-4853-0

■ 13 July

Development of a cryopreservation method of ladybird beetle ovaries

A study led by a research team at NIBB found an effective way to cryopreserve and transplant the ovaries of the multicolored Asian ladybird beetle, *Harmonia axyridis*. This study was the first to report a successful ovary cryopreservation method in a small non-model insect, or an insect that had not previously been widely studied in this manner. The team successfully preserved ladybird beetle ovaries by first slowly cooling them down to -80°C in a freezing container at a rate of 1°C per minute. The frozen ovaries were then placed into liquid nitrogen, where they were stored until further use. Immediate thawing at 37°C was done at the time of transplant, and thawed donor ovaries were then placed in the recipient larva's body (see p. 55).

Kawaguchi, H., and Niimi, T. (2018). A method for cryopreservation of ovaries of the ladybird beetle, *Harmonia axyridis*. J. Insect Biotechnol. Sericol. *87*, 35-44. doi: 10.11416/jibs.87.2_035

■ 5 September

Cell-to-cell heterogeneity in molecular activity leads to cell fate decisions regarding whether they will live or die: variation of negative regulation between cells leads to heterogeneity

When cells of multicellular organisms suffer from stress and are irretrievably damaged, apoptosis (cell death) is actively induced and cells 'commit suicide' so to speak. Once the switch of apoptosis is turned on, cell fate is irreversibly determined. The switch of apoptosis is known to include stress-activated protein kinases (SAPKs) such as p38 and JNK, but its detailed mechanism has been unknown. NIBB researchers observed the activities of p38 and JNK in individual live cells under various stresses, and found that JNK is negatively regulated by p38. Furthermore, it was discovered that the variation of this negative regulation between cells leads to the variation of JNK activity. They also confirmed that an above-threshold JNK activity in individual UV-stressed cells leads to apoptotic cell deaths (see p. 17).

Miura, H., Kondo, Y., Matsuda, M., and Aoki, K. (2018). Cell-to-cell heterogeneity in p38-mediated cross-inhibition of JNK causes stochastic cell death. Cell Reports *24*, 2658-2668. doi: 10.1016/j.celrep.2018.08.020

■ 21 September

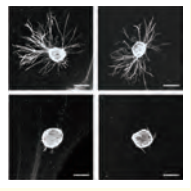
A mechanism of color pattern formation in ladybird beetles

Many ladybirds have attractive red and black color patterns. This color pattern is thought to be used a warning to predators that they are unpalatable. A research team from NIBB has identified a single gene, *pannie*r, that regulates such highly diverse ladybird color patterns found in the Asian ladybird beetle *Harmonia axyridis* which lives mainly in Siberia and East Asia, and shows >200 color patterns within a species. *pannier* was expressed in the black pigmented regions where the red pigment was not deposited. Functional inhibition of the *pannier* gene during pupal development resulted in the loss of the black color patterns and ectopic red pattern formation in the forewing (see p. 55).

Ando, T., Matsuda, T., Goto, K., Hara, K., Ito, A., Hirata, J., Yatomi, J., Kajitani, R., Okuno, M., Yamaguchi, K., Kobayashi, M., Takano, T., Minakuchi, Y., Seki, M., Suzuki, Y., Yano, K., Itoh, T., Shigenobu, S., Toyoda, A., and Niimi, T. (2018). Repeated inversions within a *pannier* intron drive diversification of intraspecific colour patterns of ladybird beetles. Nat. Commun. *9*, 3843. doi: 10.1038/s41467-018-06116-1

■ 25 September

Identification of enzymes important for the visual circuit formation in mice: the control of retinal axonal projections by PTPRJ

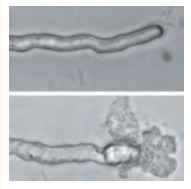


It is well known that Eph receptor-type protein tyrosine kinases play crucial roles in the formation of visual circuits. NIBB researchers previously reported that protein tyrosine phosphatase R3 subfamily members (PTPRB, PTPRH, PTPRJ, and PTPRO) dephosphorylated Eph receptors in cultured cells. In the present study, they revealed that *Ptprj*-knockout mice exhibited abnormal projections of retinal axons through the enhancement of the Eph receptor activity. They also showed that PTPRJ inhibited a non-receptor-type tyrosine kinase, c-Abl, a downstream target of Eph signaling in retinal axons. PTPRJ thus regulates retinal axonal projections by inhibiting Eph receptors and c-Abl (see p. 42).

Yang, Y., Shintani, T., Takeuchi, Y., Shirasawa, T., and Noda, M. (2018). Protein tyrosine phosphatase receptor type J (PTPRJ) regulates retinal axonal projections by inhibiting Eph and Ab1 kinases in mice. J. Neurosci. *38*, 8345-8363. doi: 10.1523/JNEUROSCI.0128-18.2018

■ 1 October

Discovery of a mechanism of membrane transport that is necessary in elongating pollen tubes

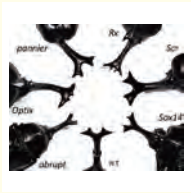


During plant reproduction, sperm cells are delivered to ovules through elongating pollen tubes. This elongation requires several receptor proteins such as ANXUR being localized at the tip of the pollen tube. However, the mechanism of localization had been unknown to date. To this end, NIBB researchers found molecules (PICALM5a and PICALM5b) required for this localization. The molecules belong to ANTH proteins related to the transport of membrane embedded proteins and play a role in loading ANXUR protein to membrane vesicles at the sub-apical region of the pollen tube. The molecules were not necessary in loading another receptor protein necessary for pollen tube guidance. The diversification of the functions of ANTH proteins was shown to be closely related to the evolution of reproduction mechanisms in plants (see p. 14).

Muro, K., Matsuura-Tokita, K., Tsukamoto, R., Kanaoka, MM., Ebine, K., Higashiyama, T., Nakano, A., and Ueda, T. (2018). ANTH domain-containing proteins are required for the pollen tube plasma membrane integrity via recycling ANXUR kinases. Commun. Biol. *1*, 152. doi: 10.1038/s42003-018-0158-8

■ 5 October

The identification of the genes necessary in horn formation in rhinoceros beetles

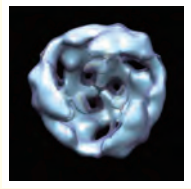


Many are fascinated by rhinoceros beetles because of their spectacular horns but the genes related to the formation of these horns have been unknown. NIBB researchers have identified eleven genes related to horn formation by extensive genome analyses using next-generation DNA sequencers. Inhibition of these genes resulted in the disappearance, shortening, or malformation of the horn or the formation of excessive horns. The comparison of these genes and already reported horn formation genes in dung beetles suggested the presence of a common mechanism between the two beetle groups. These results are important in understanding the evolutionary process of the beetle horn (see p. 55).

Ohde, T., Morita, S., Shigenobu, S., Morita, J., Mizutani, T., Gotoh, H., Zinna, R.A., Nakata, M., Ito, Y., Wada, K., Kitano, Y., Yuzaki, K., Toga, K., Mase, M., Kadota, K., Rushe, J., Lavine, L.C., Emlen, D.J., and Niimi, T. (2018). Rhinoceros beetle horn development reveals deep parallels with dung beetles. PLoS Genet. *14*, e1007651. doi: 10.1371/journal.pgen.1007651

■ 12 October

Determining the diffusion range of information by assembly and dissociation of the Wnt protein complex: how information is distributed among the society of cells



Cell-to-cell communication is indispensable in the formation and maintenance of tissues and organs made up of many cells. Signal proteins such as Wnt are secreted by specific cells in the tissue and received by surrounding cells thereby establishing cell-to-cell communication. How the diffusion range of the Wnt is determined has been unknown. NIBB researchers found that the Wnt proteins excreted outside of the cell are assembled into high-molecular-weight complexes whose smallest unit is the trimer of the Wnt protein. They also proposed a mechanism that the balance between assembly and dissociation of the Wnt protein complex determines the range of diffusion of the Wnt signal (see p. 33).

Takada, R., Mii, Y., Krayukhina, E., Maruyama, Y., Mio, K., Sasaki, Y., Shinkawa, T., Pack, C.-G., Sako, Y., Sato, C., Uchiyama, S., and Takada, S. (2018). Assembly of protein complexes restricts diffusion of Wnt3a proteins Commun. Biol. *1*, 165. doi: 10.1038/s42003-018-0172-x

■ 16 October

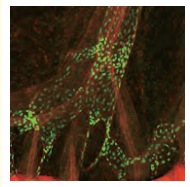
Firefly genomes disclosed the evolution of bioluminescence genes in fireflies

The genome of the Japanese firefly, *Aquatica lateralis*, was decoded at NIBB, and was compared with the genome of the North American firefly, *Photinus pyralis*, and the genome of a Caribbean click beetle, *Ignelater luminosus*, both decoded by researchers listed among those found in the reference list. The light of fireflies is emitted by the reaction of an enzyme called luciferase and the substrate called luciferin. The genome comparison showed that the luciferase gene has evolved from the acyl-CoA synthetase gene ubiquitously found in non-light emitting organisms. The luciferase gene of the click beetle was shown to be evolved from the same gene, but the evolution was independent from that found in fireflies (see p. 98 and 81).

Fallon, T.R., Lower, S.E., Chang, C.-H., Bessho-Uehara, M., Martin, G.J., Bewick, A.J., Behringer, M., Debat, H.J., Wong, I., Day, J.C., Suvorov, A., Silva, C.J., Stanger-Hall, K.F., Hall, D.W., Schmitz, R.J., Nelson, D.R., Lewis, S., Shigenobu, S., Bybee, S.M., Larracuente, A.M., Oba, Y., and Weng, J.-K. (2018). Firefly genomes illuminate parallel origins of bioluminescence in beetles. eLife *7*, e36495. doi: 10.7554/eLife.36495

■ 27 November

Lymphatic valve formation requires mechanosensor channel PIEZO1

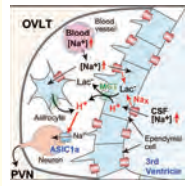


Valves are essential within the cardiac and lymphatic systems, ensuring proper blood and lymph circulation. However, little is known how they are formed within the body. Following up previous research that showed that human patients with *PIEZO1* loss-of-function mutations are associated with congenital lymphedema, a team led by biologists at The National Institute for Basic Biology (NIBB), The Scripps Research Institute and Howard Hughes Medical Institute, USA, discovered through the analysis of mouse models lacking PIEZO1 (a mechanically activated cation channel in endothelial cells) that lymphatic valve formation requires the abovementioned cation channel (see p. 36).

Nonomura, K., Lukacs, V., Sweet, D.T., Goddard, L.M., Kanie, A., Whitwam, T., Ranade, S.S., Fujimori, T., Kahn, M.L., and Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc. Natl. Acad. Sci. USA *115*, 12817-12822. doi: 10.1073/pnas.1817070115

■ 30 November

Elucidation of central mechanisms of salt-induced hypertension through activation of sympathetic nerve activities

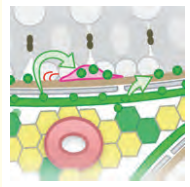


Hypertension is a major risk factor for cardiovascular diseases worldwide, and a positive correlation between salt intake and blood pressure has long been postulated. However, underlying mechanisms responsible for [Na^+] sensing and signaling pathways to induce sympathetically mediated blood pressure elevations have yet to be elucidated. An NIBB research team has previously reported that Na_x channels expressed in specific glial cells in the sensory circumventricular organs (SFO and OVLT) are the sensor for monitoring of increases in [Na^+] in body fluids to control salt/water-intake behaviors. They have now found that sympathetic activation leading to blood pressure increases was not induced in *Na_x*-knockout mice by mandatory high salt intakes or the intraperitoneal/intracerebroventricular infusions of hypertonic NaCl solutions. The study showed that Na_x channels in the OVLT function as the brain Na^+-level sensor for blood pressure control. The research team further demonstrated that the Na_x signal is transferred from glial cells to OVLT (→PVN) neurons harboring acid-sensitive ion channels (ASIC1a) by using H^+ as a gliotransmitter (see p. 42).

Nomura, K., Hiyama, T.Y., Sakuta, H., Matsuda, T., Lin, C.-H., Kobayashi, K., Kobayashi, K., Kuwaki, T., Takahashi, K., Matsui, S., and Noda, M. [Na^+] increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASIC1a. Neuron 2018 Nov 29. doi: 10.1016/j.neuron.2018.11.017

■ 21 December

How sperm stem cells maintain their number



Steady sperm production relies on a constant number of sperm stem cells in the testis. Regarding this phenomenon, researchers from both NIBB and the University of Cambridge in the UK revealed a novel mechanism for stem cell number control. Gained through quantitative analysis, their results showed that constant sperm stem cell numbers are achieved in mouse testes through a self-organized process in which they actively migrate and compete for a limited supply of self-renewal-promoting fibroblast growth factors (FGFs). In this study, the researchers found that a subset of lymphatic endothelial cells produce FGFs which promote stem cell self-renewal. The research illustrated a competitive scenario where stem cells that consumed more FGFs were likely to duplicate, while those that consumed less were inclined to differentiate (see p. 39).

Kitadate, Y., Jörg, D.J., Tokue, M., Maruyama, A., Ichikawa, R., Tsuchiya, S., Segi-Nishida, E., Nakagawa T., Uchida, A., Kimura-Yoshida, C., Mizuno, S., Sugiyama, F., Azami, T., Ema, M., Noda, C., Kobayashi, S., Matsuo, I., Kanai, Y., Nagasawa, T., Sugimoto, Y., Takahashi S., *Simons, B.D., and *Yoshida, S. Competition for mitogens regulates spermatogenic stem cell homeostasis in an open niche. Cell Stem Cell. 2018 Dec 20. (*: Co-corresponding authors) doi: 10.1016/j.stem.2018.11.013

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	KIYOHARA, Megumi

Membrane traffic between single membrane-bounded organelles plays an integral role in various cell activities in eukaryotic cells. Recent comparative genomics have indicated that membrane trafficking pathways are diversified among eukaryotic lineages, which is associated with the lineage-specific acquisition of new trafficking pathways and the secondary loss of preexisting trafficking routes. Our long-term goal is to reveal 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 liverwort model, *Marchantia polymorpha*. We also aim to elucidate the detailed molecular mechanisms and physiological functions of membrane trafficking in higher-ordered plant functions.

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

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

1-1 Characterization of RAB GTPases in the liverwort, *Marchantia polymorpha*

To gain information on the diversification of membrane trafficking pathways during land plant evolution, we systematically analyzed RAB GTPases in *Marchantia polymorpha*. Comparisons of the organization of this protein family with other plant lineages, followed by 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) relocation of conserved machinery components to distinct trafficking events, and 3) secondary loss of conserved machinery components during evolution (Minamino *et al*., 2018).

1-2 Analysis of the liverwort-specific organelle: the 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) whose origin and biogenesis remain unclear. We are currently characterizing membrane trafficking pathways responsible for oil body biogenesis, as well as analyzing the function of a master regulator of oil body biogenesis.

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, such as protein degradation, protein storage, and regulation of turgor pressure. To perform these vacuolar functions, a wide variety of vacuolar proteins and other components must be properly transported to the vacuole, the entirety of which is mediated by membrane trafficking. To understand the molecular mechanisms of vacuolar transport in plants, we analyzed the molecular functions of RAB5 and RAB7 in *A. thaliana* cells. A tethering complex, CORVET, is known to act as an effector of Vps21/RAB5 in regulating endosomal transport in yeast and animal cells. CORVET subunits are also conserved in *A. thaliana*, but the molecular function of CORVET remains unclear. We found that VPS3, one of the CORVET subunits, acts in the RAB5-dependent and RAB7-independent vacuolar transport pathway in *A. thaliana*. 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 (Figure 1). These findings demonstrated that the plant vacuolar transport system has diverged from vacuolar/lysosomal transport systems in non-plant systems (Takemoto *et al*., 2018).

We also conducted detailed analyses of vacuolar SNARE proteins. Defective functions of vacuolar SNAREs affect both vacuolar transport and morphology. The *sgr3-1* (*shoot gravitropism3*) mutant was isolated as a mutant that is defective in shoot gravitropism, which resulted from a point

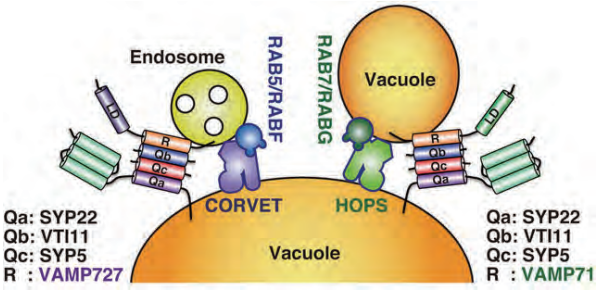

Figure 1. Schematic illustration of tethering-fusion modules acting in vacuolar transport in *A. thaliana* cells (Takemoto *et al*., 2018).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

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* may be a useful means of examining the functions of the vacuolar SNARE. We are exploring vacuolar dynamics regulated by SYP22 by analyzing the effect of the *sgr3-1* mutation in a more detailed manner.

1-4 Integration of two RAB5 groups in plants

RAB5 is a member of RAB GTPase acting in endosomal transport in eukaryotic cells that has existed within almost all eukaryotic organisms, as well as their common ancestor: LECA. Plants possess two different RAB5 groups, canonical and plant-unique types, which act via unknown counteracting mechanisms. We identified an effector molecule of the plant-unique RAB5 in *A. thaliana*, ARA6, which we designated as PLANT-UNIQUE RAB5 EFFECTOR 2 (PUF2). Preferential co-localization with canonical RAB5 on endosomes and genetic interaction analysis (Figure 2) indicated that PUF2 coordinates vacuolar transport with canonical RAB5, although PUF2 was identified as an effector of ARA6. Competitive binding of PUF2 with GTP-bound ARA6 and GDP-bound canonical RAB5, interacting together with the shared activating factor VPS9a, showed that ARA6 negatively regulates canonical RAB5-mediated vacuolar transport by titrating PUF2 and VPS9a. These results suggest a unique and unprecedented function for RAB effectors involving the integration of two RAB groups to orchestrate endosomal trafficking in plant cells.

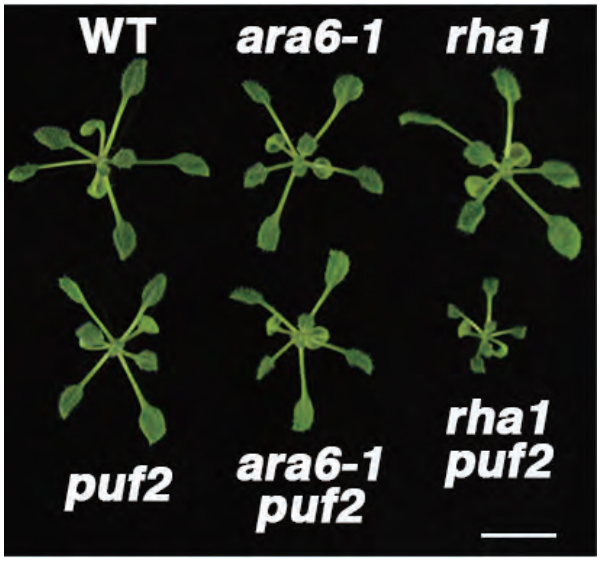

Figure 2. Genetic interactions among mutations in canonical (*RHA1*) and plant-unique (*ARA6*) *RAB5* and *PUF2* (Ito *et al*., 2018).

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

2-1 Membrane trafficking in plant gametogenesis

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

Cytokinesis in land plants is achieved by the re-direction of the secretory pathway. As such, KNOLLE/SYP111 and KEULE/SEC11 play important roles in membrane fusion at the forming cell plate in somatic cells of Arabidopsis. Conversely, no deleterious effects on gametogenesis have been reported regarding mutations in genes for these proteins thus far. We found that other SYP1 and SEC1 members are highly expressed during male gametogenesis. Analyses of the functions of these proteins during male gametogenesis are currently underway.

Distinct from seed plants, basal land plants including *M. polymorpha* utilize the spermatozoid with two (or more) motile flagella as the male gamete in sexual reproduction. We visualized the process of spermatozoid formation, especially spermiogenesis, using fluorescently-tagged organelle markers. The majority of the endomembranous organelles such as the Golgi apparatus were removed from maturing spermatozoid cells, and the plasma membrane was also reorganized during spermiogenesis. Inspection by transmission electron microscope and live-cell imaging analyses also indicated that the number of degradative organelles such as the multivesicular endosomes, vacuoles, and autophagosomes, was transiently increased during this process. To reveal the molecular mechanisms of cytoplasm removal and organelle remodeling, we are now analyzing the contribution of autophagy in these processes. Autophagy-defective mutants exhibited defects in the areas of morphogenesis, cytoplasm removal, and motility of spermatozoids, indicating the numerous crucial roles played by autophagy during spermiogenesis in *M. polymorpha*. We are also analyzing the role of RAB GTPases in flagella formation. Through a comprehensive analysis of RAB GTPases in *M. polymorpha*, we found that a RAB GTPase plays an essential role in generating fully functional flagella.

2-2 Membrane trafficking in sexual reproduction

During plant reproduction, sperm cells are delivered to ovules through growing pollen tubes. This process involves tip-localized receptor kinases regulating the integrity and/or guidance of pollen tubes, whose localizations must be strictly regulated. We found that a pair of AP180 N-terminal homology domain-containing proteins (ANTH proteins), PICALM5a and PICALM5b, are responsible for the tip-localization of ANXUR receptor kinases acting in an autocrine signaling pathway required for pollen tube integrity in *A. thaliana*. The *picalm5a picalm5b* double mutant exhibits reduced fertility, and the double mutant pollen is defective in pollen tube integrity with premature bursts. The tip localizations of ANXUR proteins are severely impaired in *picalm5a picalm5b* pollen tubes (Figure 3), whereas another receptor kinase PRK6 acting in pollen tube guidance is not affected. Based on these results, we propose that PICALM5 proteins serve as specific loading adaptors to recycle ANXUR proteins (Muro *et al*., 2018).

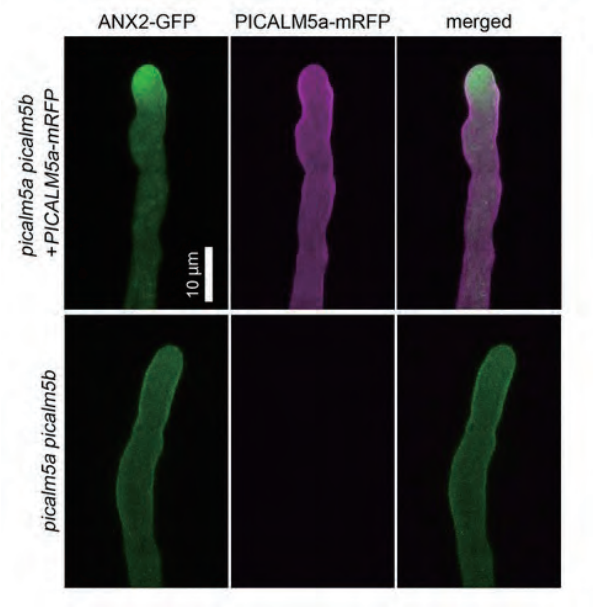

Figure 3. PICALM5s are required for tip-localization of ANX2 (Muro *et al*., 2018).

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- Cui, Y., Cao, W., He, Y., Zhao, Q., Wakazaki, M., Zhuang, X., Gao, J., Zeng, Y., Gao, C., Ding, Y., Wong, H.Y., Wong, W.S., Lam, H.K., Wang, P., Ueda, T., Rojas-Pierce, M., Toyooka, K., Kang BH., and Jiang L. A whole-cell electron tomography model of vacuole biogenesis in Arabidopsis root cells. Nature Plants 2018 Dec 17.
- Uemura, T., Nakano, T.R., Takagi, J., Wang, Y., Kramere, K., Finkemeier, I., Nakagami, H., Tsuda, K., Ueda, T., Schulze-Lefert, P., and Nakano, A. A Golgi-released subpopulation of the *trans*-Golgi network mediates constitutive and pathogen-inducible protein secretion in Arabidopsis. Plant Phys. 2018 Dec 13.

DIVISION OF QUANTITATIVE BIOLOGY

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Living cells act as input-output (I/O) units, in which their environment and/or internal states are recognized on the cell surface and processed within the cell, leading to an adaptive response to any changes that occur (Figure 1). This cellular information processing is mainly controlled by intracellular signal transduction, which is comprised of a series of chemical reactions, most commonly protein phosphorylation. Dysregulation of this process 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 a perceived increase in the pathway's complexity. Computer-assisted simulation is one of the most promising approaches to understanding 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 can be solved numerically by computers. In fact, a number of signaling pathway simulation models have been reported to date. However, most of the kinetic parameters utilized for these simulation models were not measured experimentally, but rather were assumed by fitting the experimental data with the simulation model or were simply determined arbitrarily. Consequently, there are substantial differences in the kinetic parameters between these studies, thereby making it difficult to quantitatively evaluate these simulation models.

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

I. Visualization of cell signaling

With the advent of fluorescent proteins, it has become possible to visualize kinetic reactions at the single cell level. Förster (or fluorescence) resonance energy transfer (FRET) is a non-radiative process, in which the excitation energy

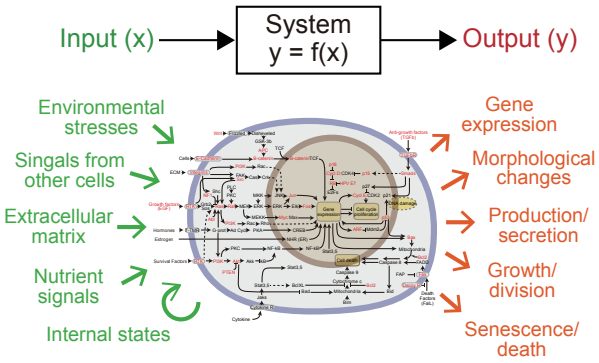

Figure 1. Information processing by intracellular signaling devices and networks.

of a donor fluorophore is transferred to a nearby acceptor fluorophore. Taking advantage of this principle, FRET-based biosensors allowed us to detect the 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). By using a FRET biosensor, we revealed the role of temporal and spatial dynamics of ERK activation in cell proliferation (Aoki K, Mol Cell, 2013) and collective cell migration (Aoki K, Dev Cell, 2017).

Although FRET biosensors enable us to monitor cell signaling events, FRET imaging is not suitable for multiplexed imaging, because these biosensors are composed of two different fluorophores in many cases. To circumvent this limitation, we are currently developing biosensors based on the

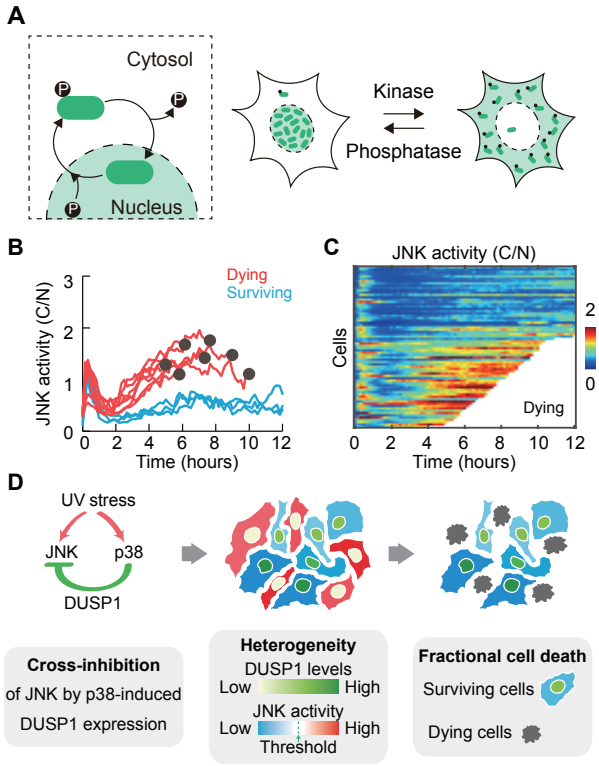

Figure 2. Multiplexed imaging of JNK and p38, stress-activating protein kinases (SAPKs) signaling. (A) Schematic representation of kinase translocation reporter (KTR). (B) Time-course of UV-C-induced JNK activation in surviving (blue) and dying (red) cells. (C) A heatmap shows time-courses of JNK activation and cell death. (D) The schematic shows p38-induced cross-inhibition through DUSP1 expression emerges JNK heterogeneity, leading to the fractional killing upon UV-C stress.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

principle of the kinase translocation reporter (KTR) system. The reporter itself is translocated from nucleus to cytoplasm when it is phosphorylated by its target kinase (Figure 2A). This approach yields several ERK and Akt kinase reporters (Maryu G, Cell Strut, Funct, 2016). We further applied KTRs for p38 and JNK, stress-activating protein kinases (SAPKs) to investigate how stress stimuli induced p38 and JNK activation, and subsequently lead to cell death. Various stresses activated JNK and p38 within various dynamics. In all cases, p38 suppressed JNK activity in a cross-inhibitory manner. We demonstrated that p38 antagonizes JNK through both transcriptional and post-translational mechanisms. This cross-inhibition generates cellular heterogeneity in JNK activity after stress exposure (Figure 2B and 2C). Our data indicates that this heterogeneity in JNK activity plays a role in fractional killing in response to UV stress (Figure 2D) (Miura H, Cell Reports, 2018).

II. Quantification of cell signaling and physical parameters

Kinetic parameters such as protein concentration and dissociation constant, Kd, have traditionally 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 *S. pombe* and mammalian cultured cells. We developed an efficient knock-in system with CRISPR/Cas9-mediated genome editing techniquescombined 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 AT, Goto Y, bioRxiv, 2018).

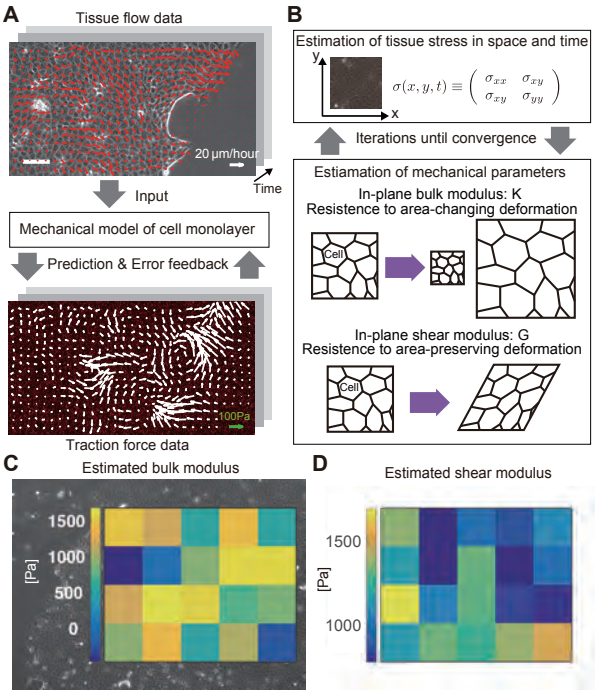

Figure 3. Overview of the inference of mechanical parameters. (A and B) Schematic representation of the inference scheme and algorithm of mechanical properties. (C and D) Spatial distribution of the estimated elastic moduli.

In order to quantitatively understand the morphogenesis of living tissues, we need to elucidate the mechanical properties which describe how the tissues form in response to self-generated forces. To this end, we proposed a non-invasive approach for the statistical estimation of the mechanical properties, by combining tissue mechanics modeling and statistical machine learning (Figure 3A and 3B). This method was applied to the collective migration of Madin-Darby canine kidney cells, where the tissue flow and force were simultaneously observed by phase contrast imaging and traction force microscopy. As a result, the estimated elastic moduli were detected in the order of kPa μm (Figure 3C and 3D). We confirmed that our elastic solid tissue model outperformed null-hypothetical models in terms of forecast accuracy for the traction force fields, indicating that mechanical dynamics are dominated by elasticity. The results validate our framework, which paves the way to estimate *in vivo* mechanical properties of tissues during morphogenesis (Kondo Y, PLoS Comp, 2018).

III. Manipulation of cell signaling

Artificial manipulation of biochemical networks may be useful in the 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. Of these two, the LID system is stronger 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 two parts of the PhyB-PIF complex dissociate from each other by infra-red light exposure (Figure 4A). The reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system, because association or dissociation only are 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 have developed a method for synthesis of PCB in mammalian cells by introduction of the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into mitochondria (Uda Y, PNAS, 2017) (Figure 4B).

To take full advantage, we applied the genetically encoded PCB synthesis system to fission yeast *S. pombe* and *C. elegans*, of which both experienced difficulty in taking up externally-delivered PCB. As we expected, the expression of *HO1*, *PcyA*, *Fd*, and *Fnr* genes in *S. pombe* increased PCB synthesis, allowing us to manipulate subcellular distribution of proteins by light (Figure 4C). *C. elegans* demonstrated uptake of externally delivered PCB only in the gut, but not in the muscles or neurons (Figure 4D), while the introduction of *HO1*, *PcyA*, *Fd*, and *Fnr* genes enabled to reconstitute PCB synthesis in the muscles, guts and neurons (Figure 4D and 4E). In addition to this, we were able to demonstrate light-

induced PhyB-PIF hetero-dimerization in *C. elegans* for the first time (Figure 4F). 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.

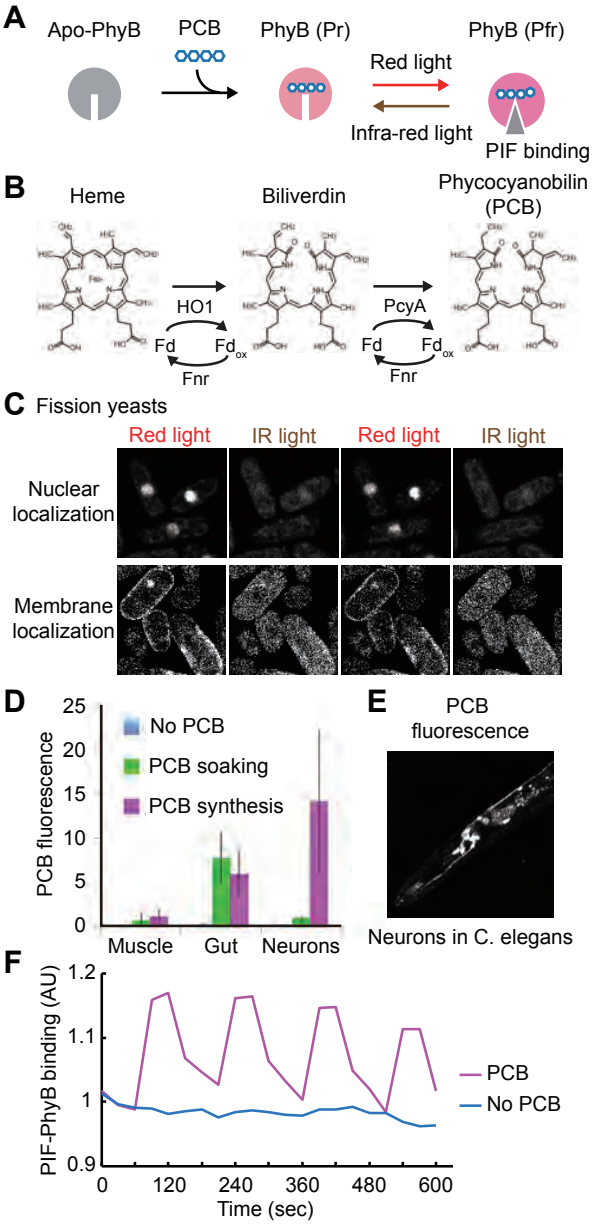

Figure 4. PhyB-PIF light-inducible dimerization (LID) system and its application to the manipulation of cell signaling. (A) Apo-PhyB covalently attaches its chromophore, which is phycocyanobilin (PCB) to produce holo-PhyB. There are two holo-PhyB forms, PhyB (Pr) and PhyB (Pfr), which change over in manner dependent on the chromophore status. Only PhyB (Pfr) associates with PIF. (B) The metabolic pathway of phytochrome chromophores, PCB. (C) Light-induced translocation to nucleus (upper) and plasma membrane (lower) of PIF3-EGFP protein in fission yeasts expressing PcyA, HO1, Fd, and Fnr. (D) *C. elegans* was soaked with DMSO (blue) or PCB (green), or introduced by PcyA, HO1, Fd, and Fnr genes to synthesize PCB (magenta). PCB fluorescence was quantified. (E) A representative image of PCB fluorescence in neurons of C. elegans. (F) Light-induced PhyB-PIF binding in gut of *C. elegans*.

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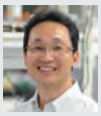
DIVISION OF CHROMATIN REGULATION



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The stable inheritance of gene expression or repression states 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 structures has been linked to the covalent modifications of histone tails, chromatin-associated proteins, and non-coding RNAs. However, the exact means by which such chromatin-based epigenetic information is established and faithfully maintained across cell divisions and throughout development remains unclear. To try to gain a better understanding of the molecular mechanisms underlying chromatin-based epigenetic phenomena, our lab uses mammalian culture cells, fission yeast, *Schizosaccharomyces pombe*, and ciliate *Tetrahymena* as model systems for studying the molecular mechanisms of higher-order chromatin assembly. We are also attempting to determine the cellular functions of chromatin modifying factors so that we can develop a better understanding of complicated epigenetic phenomena in higher eukaryotic cells.

I. Establishment and maintenance of higher-order chromatin structures

In eukaryotic cells, the assembly of higher-order chromatin structures, 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 structures. However, the mechanism by which HP1 folds chromatin containing H3K9me into a higher order structure has not been elucidated yet. To understand the structural basis of heterochromatin, we prepared a dinucleosome containing H3K9me. The HP1α-dinucleosome complexes containing H3K9me were fixed with glutaraldehyde and subjected to a cryo-EM analysis. The HP1α-dinucleosome complexes were visualized in cryo-EM images (Figure 1A), particles were selected semi-automatically, and 2D class averages were obtained by unsupervised maximum likelihood classification (Figure 1B). This was followed by 3D reconstruction and classification. In the cryo-EM structure of the HP1α-dinucleosome complexes, the two nucleosomes do not directly interact with each other, and the HP1α dimer binds to two neighboring nucleosomes (Figure 1C). We ultimately confirmed that the treatment of micrococcal nuclease (MNase) preferentially cleaved the linker DNA, but not the nucleosomal DNA. The present structure of the HP1α-dinucleosome complexes matches best to the nucleosome-bridging model, in which two neighboring nucleosomes are connected by the HP1 dimer, and depicts the fundamental architecture of heterochromatin.

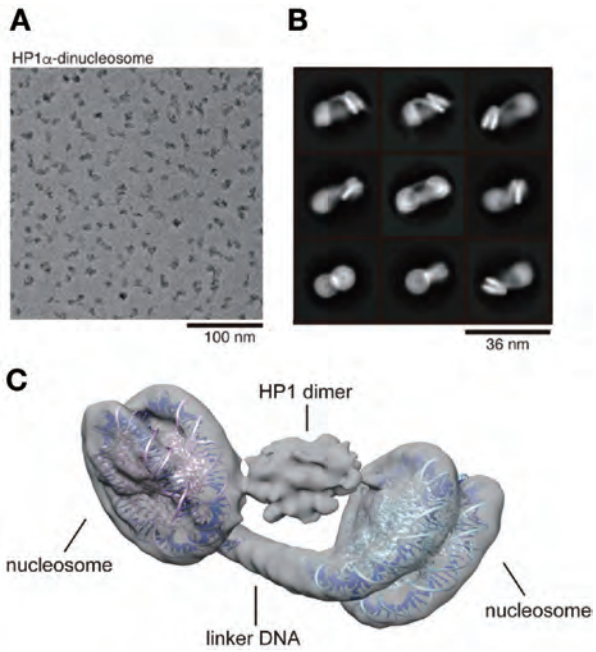

Figure 1. The structure of HP1α-dinucleosome complex. (A) Digital micrograph of HP1α-dinucleosome particles in amorphous ice; recorded in-focus with a Volta phase plate. Scale bar: 100 nm. (B) Selected 2D class average of 200 classes from 187,784 aliged single-particle images of the HP1α-dinucleosome complex. Box size: 36 nm. (C) Representation of the reconstituted three-dimensional electron potential of the HP1α-dinucleosome complex.

II. Roles 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. To examine the roles played by HP1α's mitotic phosphorylation, we determined HP1α's mitotic phosphorylation sites and the cellular behavior of HP1α with mitotic phosphorylation. We found that S92 in the hinge region is the main mitotic phosphorylation site in human HP1α and that HP1α's S92 phosphorylation (S92ph) was regulated by

Aurora B kinase (AURKB) and two serine/threonine phosphatases. Immunoblotting analysis using cell cycle-synchronized cells demonstrated that HP1α S92ph precedes H3S10 phosphorylation; a major hallmark of mitotic chromatin. In addition, chromatin fractionation analyses revealed that hinge region-phosphorylated HP1α was preferentially dissociated from mitotic chromatin (Figure 2A). Furthermore, EMSA assays demonstrated that AURKB-mediated phosphorylation contributed to a decrease in HP1α's DNA-binding activity (Figure 2B). Although HP1α's mitotic behavior was previously linked to H3 serine 10 phosphorylation, which blocks the binding of HP1α's CD to H3K9me, our findings suggest that mitotic phosphorylation in HP1α's hinge region also contributes to changes in HP1α's association with mitotic chromatin.

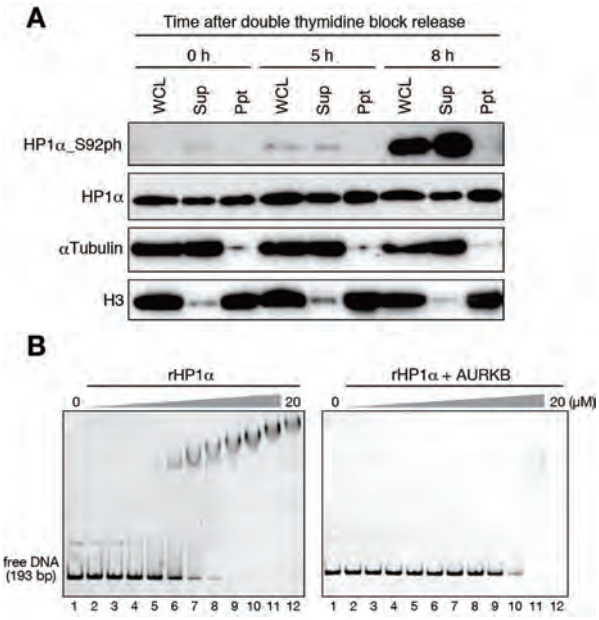

Figure 2. Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding. (A) Chromatin fractionation assays were performed using synchronized RPE-1 cells. Whole cell lysates (WCLs) and soluble (Sup) and insoluble chromatin-enriched (Ppt) fractions were resolved by SDS-PAGE and analyzed by immunoblotting. (B) Standard results of EMSAs that were performed with control or phosphorylated HP1α. Various concentrations of the mutant HP1α were incubated with 193-bp 601 DNA. The protein-DNA complexes were analyzed by 5% native-PAGE and SYBR Gold staining.

2-2 The binding of Chp2's CD to methylated H3K9 is essential for Chp2's role in heterochromatin assembly in fission yeast

The binding of HP1 to H3K9me is an essential step in heterochromatin assembly. Chp2, an HP1-family protein in the fission yeast *Schizosaccharomyces pombe*, is required for heterochromatic silencing. Chp2 recruits SHREC, a multifunctional protein complex containing the nucleosome remodeler Mit1 and the histone deacetylase Clr3 to perform this function. Although the targeting of SHREC to chromatin is thought to occur via two distinct modules regulated by the SHREC components Chp2 and Clr2, it is not clear how Chp2's chromatin binding regulates SHREC function. To investigate the role of H3K9me binding in Chp2's function, we created a strain that expressed a mutated Chp2 protein containing W199A (Chp2-W199A, in which tryptophan 199, one of the three conserved residues recognizing H3K9me, was changed to alanine). We then examined its effect on Chp2's silencing function and for SHREC's targeting to chromatin. Cells expressing Chp2-W199A have a silencing defect, with a phenotype similar to that of *chp2*-null cells (Figure 3). Genetic analysis using a synthetic silencing system revealed that a Chp2 mutant and SHREC-component mutants had similar phenotypes, suggesting that Chp2's function also affects SHREC's chromatin binding. Size-exclusion chromatography of native protein complexes showed that Chp2-CD's binding of H3K9me ensures Clr3's chromatin binding, and suggested that SHREC's chromatin binding is mediated by separable functional modules. Interestingly, we found that the stability of the Chp2 protein depended on the Clr3 protein's histone deacetylase activity. Our findings demonstrate that Chp2's H3K9me binding is critical for SHREC function and that the two modules within the SHREC complex are interdependent.

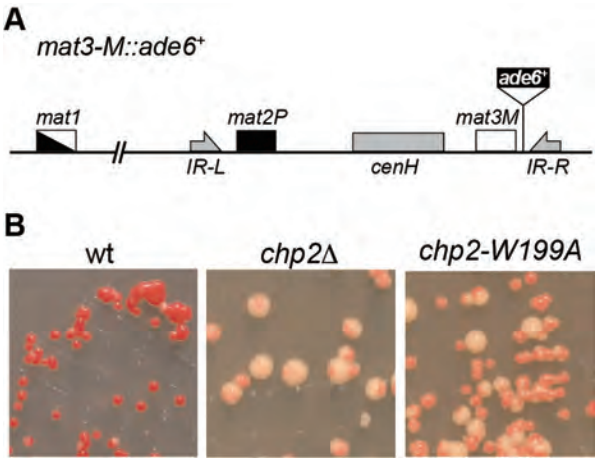

Figure 3. Chp2's H3K9me-binding ability is essential for its function in heterochromatin formation. (A) Schematic of the mating-type (*mat*) locus, showing the position of the silencing-reporter gene (*mat3-M::ade6*$^+$). (B) Wild-type and mutant strains with an *ade6*$^+$reporter gene inserted into the mating-type region were streaked on medium containing low amount of adenine (YE medium).

III. A proteomic approach to identifying chromatin-bound RNA-binding proteins

Various coding and non-coding transcripts are known to associate with chromatin and now there is accumulating evidence that interaction between RNA-binding proteins (RBPs) and RNA molecules regulate not only co-transcriptional mRNA processing, but also other biological processes within the nucleus. Although over a thousand RBPs have been identified through several mass spectrometry-based methods, it is still unclear which of these RBPs actually associate with chromatin, especially through interaction with RNAs. In addition, biological outcomes of such RBP-RNA-chromatin interactions are yet to be elucidated. In order to grasp the whole picture of physical or functional interactions between RNAs and RBPs in the nucleus, we have developed a simple proteomics-based method for systematic screening of RBPs that are anchored to chromatin and/or insoluble nuclear substructures by RNA molecules. We used RNase A to release said RBPs from the chromatin fraction (Figure

4A) and analyzed 'RNase A-solubilized' proteins by mass spectrometry. Using this method, we were able to identify 156 RNase A-solubilized proteins of which 144 were known RBPs/RBP candidates. Interestingly, several key players of the non-homologous end-joining (NHEJ) pathway were enriched in RNase A-solubilized fraction (Figure 4B) and the RNA-mediated chromatin association of these factors appeared to be dependent on transcriptional elongation. Furthermore, some enzymes involved in metabolic pathways were also released from chromatin and/or an insoluble nuclear structure by RNase A treatment. In summary, our methodology is highly versatile and is potentially a useful tool to unravel new biological functions for RBP-RNA-chromatin interactions.

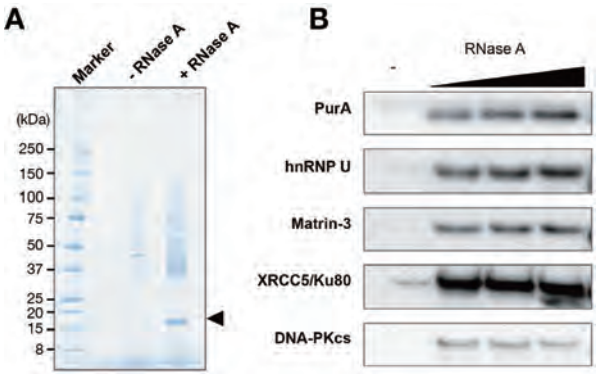

Figure 4. Proteomic analysis of RNA-dependent chromatin association of nuclear proteins. (A) Proteins solubilized after RNase A treatment were resolved by SDS-PAGE and visualized by staining with GelCode Blue dye. The black arrow indicates the position of RNase A. (B) A selection of chromatin-associated RBP candidates identified by mass spectrometry were subjected to western blot analysis.

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

Tetrahymena, a unicellular protozoan, shows nuclear dimorphism, and contains the transcriptionally silent, germline micronucleus (MIC) and the transcriptionally active, somatic macronucleus (MAC) in a single cell. When the somatic MAC differentiates from the germline MIC, approximately 12,000 transposable element (TE)-related sequences are eliminated. In this process, TEs are heterochromatinized by the pathway related to RNAi/piRNA silencing and the heterochromatin acts as a signpost for their elimination. Although previous studies have identified more than 20 heterochromatin specific components, it remains unclear how they orchestrate DNA elimination. Using a yeast two-hybrid system, we analyzed the interactions between all known heterochromatin components and all predicted HP1 proteins, which recognize methylated histones and play important roles in heterochromatin formation. This attempt identified two protein complexes regulating the phosphorylation cycle of HP1-like protein Pdd1p, that is required for the assembly of multiple heterochromatin loci used to form heterochromatin bodies (Figure 5). Ectopic tethering of proteins composing Pdd1p phosphor-regulation complexes induced DNA elimination, thus indicating that the higher order heterochromatin assembly controlled by phosphorylation cycle of Pdd1p plays a central role in DNA elimination.

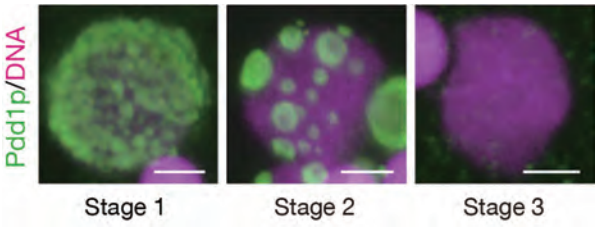

Figure 5. Three stages of heterochromatin body formation (stage 1. pre-heterochromatin body, stage 2. heterochromatin body, stage 3. post-heterochromatin body) visualized by Pdd1p (green) and DAPI (magenta). The scale bars represent 2 μm.

Publication List:

〔Original papers〕

- Hountondji, C., Crechet, J.B., Tanaka, M., Suzuki, M., Nakayama, J., Aguida, B., Bulygin, K., Karpova, G., and Baouz, S. (2018). Ribosomal protein eL42 contributes to the catalytic activity of the yeast ribosome at the elongation step of translation. Biochimie *158*, 20-33.
- Machida, S., Takizawa, Y., Ishimaru, M., Sugita, Y., Sekine, S., Nakayama, J., Wolf, M., and Kurumizaka, H. (2018). Structural basis of heterochromatin formation by human HP1. Mol. Cell *69*, 385-397.
- Maksimov, V., Oya, E., Tanaka, M., Kawaguchi, T., Hachisuka, A., Ekwall, K., Bjerling, P., and Nakayama, J. (2018). The binding of Chp2's chromodomain to methylated H3K9 is essential for Chp2's role in heterochromatin assembly in fission yeast. PLoS ONE *13*, e0201101.
- Nakayama, N., Sakashita, G., Nariai, Y., Kato, H., Shinmyozu, K., Nakayama, J., Kyo, S., Urano, T., and Nakayama, K. (2018). Cancer-related transcription regulator protein NAC1 forms a protein complex with CARM1 for ovarian cancer progression. Oncotarget *9*, 28408-28420.
- Okazaki, K., Kato, H., Iida, T., Shinmyozu, K., Nakayama, J., Murakami, Y., and Urano, T. (2018). RNAi-dependent heterochromatin assembly in fission yeast *Schizosaccharomyces pombe* requires heat-shock molecular chaperones Hsp90 and Mas5. Epigenetics Chromatin *11*, 26.

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

- Bommi, J.R., Prasada, Rao H.B.D.P., Challa, K., Higashide, M., Shinmyozu, K., Nakayama, J., Shinohara, M., and Shinohara, A. Meiosis-specific cohesion component, Rec8, promotes the localization of Mps3 SUN domain protein on the nuclear envelope. Genes Cells 2018 Dec 12.
- Nishibuchi, G., Machida, S., Nakagawa, R., Yoshimura, Y., Hiragami-Hamada, K., Abe, Y., Kurumizaka, H., Tagami, H., and Nakayama, J. Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding. J. Biochem. 2018 Dec 24.

LABORATORY OF CELL RESPONSES

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Cells sense the environment around them (*e.g.* the amount of nutrients and hormones present, 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 ambient conditions. Meiosis is essential for bringing forth genetically diverse progeny. In our laboratory, we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis (Figure 1), to research the mechanisms by which cells switch from mitosis (a kind of cell division that divides cells equally to create two identical cells) to meiosis.

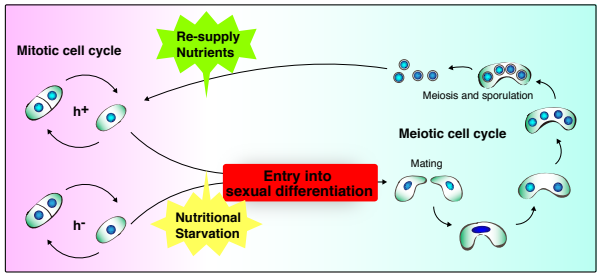

Figure 1. Life cycle of the fission yeast *S. pombe*. *S. pombe* cells proliferate by mitotic growth under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. Resulting 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 cell cycle mode from mitotic to meiotic. We focus on a highly conserved kinase, namely Target of rapamycin (TOR) kinase, which plays a key role in the recognition of nutrition and the onset of sexual differentiation in *S. pombe*. TOR kinase forms two types of complexes, TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential in suppressing sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for the onset of sexual differentiation under nitrogen starvation.

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich medium at restrictive temperatures. To gain an insight into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions, as *tor2* mutants do. We designated these mutants as *hmt*, standing for hypermating and temperature-sensitive growth. We cloned the responsible genes and found that five of the eight responsible gene encoded tRNA-related factors. The *hmt1* and *hmt2* genes encoded aminoacyl-tRNA synthetases for asparagine and proline, respectively. The *hmt3* gene encodes tRNA adenosine-34 deaminase. The *hmt4* is identical to *rpc34*, which encodes a subunit of RNA polymerase III. The *hmt5* is identical to *sfc4*, which encodes a subunit of the RNA polymerase III-specific general transcription factor IIIC. In the *hmt1-5* mutants, TORC1 activity is downregulated, suggesting that the products of these *hmt* genes may function upstream of TORC1. We also found that expression of tRNA precursors decreases upon nitrogen starvation. Furthermore, overexpression of tRNA precursors prevents TORC1 downregulation upon nitrogen starvation and represses the initiation of sexual differentiation. Based on these observations, we have proposed that tRNA precursors act as key signaling molecules in the TORC1 pathway in response to nitrogen availability (Figure 2).

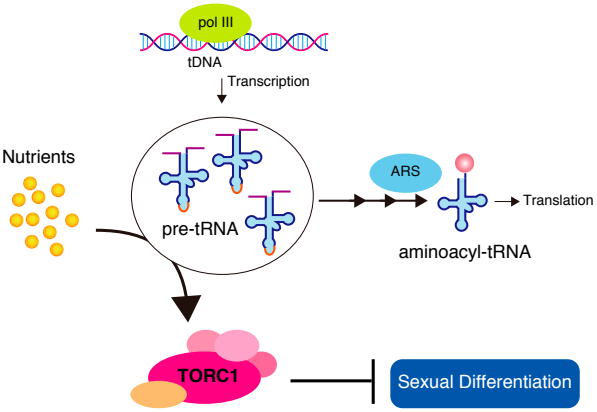

Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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

The expression of hundreds of genes is 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 the formation of facultative hetero-

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

chromatin at a subset of its target genes. Furthermore, 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.

When *S. pombe* cells undergo 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.

We have shown that Mmi1 prevents untimely expression of meiotic proteins by tethering their mRNAs to nuclear foci. Mmi1 interacts with itself with the assistance of Erh1. Mmi1 self-interaction is crucial for nuclear foci formation, target transcripts elimination, their nuclear retention, and protein expression inhibition. Multi-layered suppression of meiotic genes by Mmi1 is vital for mitotic growth (Figure 3).

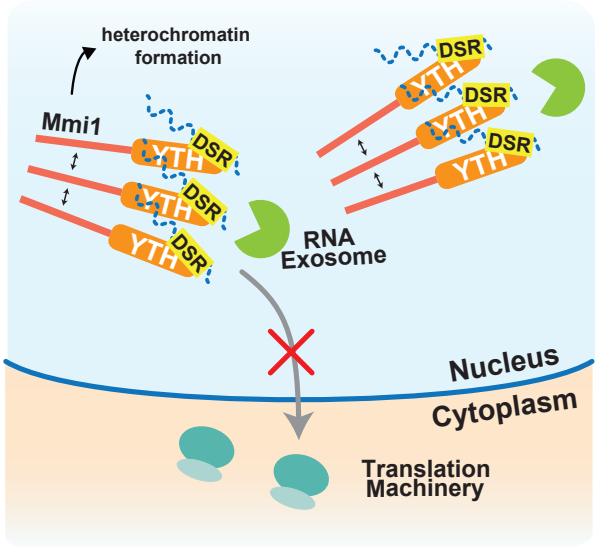

Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear export and ectopic protein expression of its targets.

Publication List:

〔Original papers〕

- Otsubo, Y., Matsuo, T., Nishimura, A., Yamamoto., M., and Yamashita, A. (2018). tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway. EMBO Rep. *19*, e44867.
- Shichino, Y., Otsubo, Y., Kimori, Y., Yamamoto., M., and Yamashita, A. (2018). YTH-RNA-binding protein prevents deleterious expression of meiotic proteins by tethering their mRNAs to nuclear foci. eLife *7*, e32155.

LABORATORY OF NEURONAL CELL BIOLOGY

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The transport of specific mRNAs and local control of translation in neuronal dendrites are part of 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 understanding 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, which mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1). We are researching the mechanism of RNA granule assembly, 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 relationship to the formation of synapses and neural networks, memory, learning, and behavior.

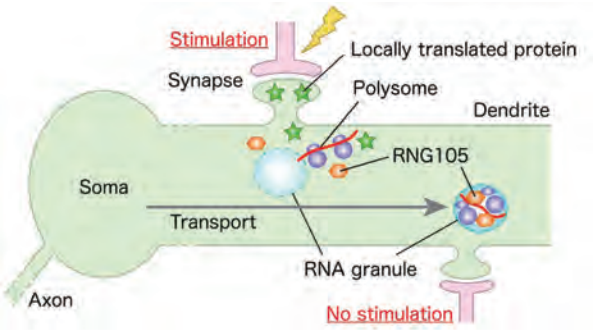

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Liquid and solid-phase RNA granules form through specific proteins and combine into biphasic granules

RNA granules consist of membrane-less RNA–protein assemblies and contain dynamic liquid-like shells and stable solid-like cores, which are thought to function in numerous processes in mRNA sorting and translational regulation (Figure 2). Abnormalities in RNA granule dynamics are associated with neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), in which solidification and aggregation of RNA granule components are facilitated in neurons. However, how these distinct liquid-like and solid-like substructures are formed, whether they are assembled by different scaffolds, and whether different RNA granule scaffolds induce these different substructures remains unknown.

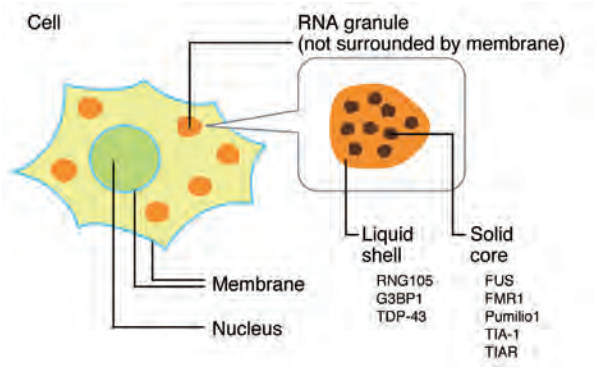

Figure 2. Membrane-less RNA granules contain liquid-like and solid-like substructures. Liquid-phase granules induced by RNG105, G3BP1, and TDP-43, and solid-like granules induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR, combine into biphasic RNA granules.

We expressed 8 kinds of RNA granule scaffold proteins (scaffolds) as GFP and RFP-tagged proteins in cultured cells, and conducted fluorescence microscopy-based morphological and molecular-dynamics analyses (Shiina, N., J. Biol. Chem. doi: 10.1074/jbc.RA118.005423). These analyses demonstrated that the scaffolds can be largely classified into two groups, liquid and solid types, which induce the formation of liquid-like and solid-like granules, respectively, when expressed separately in cultured cells. Liquid-like granules were induced by RNG105 (also known as Caprin1), G3BP1, and TDP-43, whereas solid-like granules were induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR (Figure 2). Furthermore, we found that when co-expressed, the liquid-type and solid-type scaffolds combine and form individual liquid- and solid-like substructures in the same granules (Figure 3). The combination of the different types of scaffolds reduced the immobile fractions of the solid-type scaffolds and their dose-dependent ability to inhibit translation in granules, but had little effect on the dynamics of the liquid-type scaffolds or their dose-dependent ability to increase translation in granules. These results suggest that liquid- and solid-type scaffolds form different substructures in RNA granules and the relative effect of each type on their scaffold counterpart varied. These findings provide a detailed insight into the assembly mechanism and distinct dynamics and functions of core and shell substructures in RNA granules.

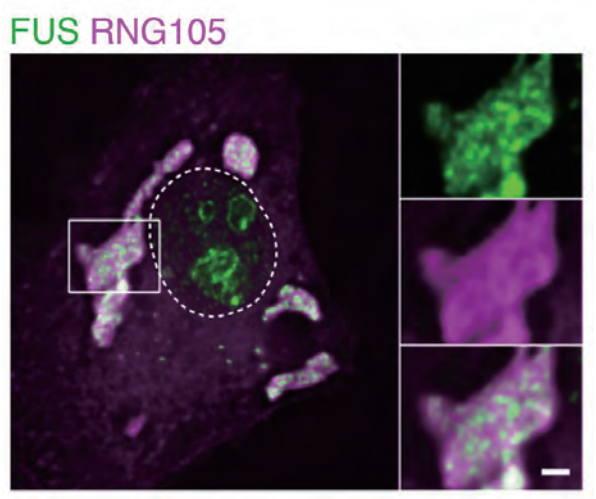

Figure 3. Liquid-type scaffolds (*e.g.* RNG105) and solid-type scaffolds (*e.g.* FUS) combine into the same granules and form individual liquid-like and solid-like substructures,. The panels on the right are magnified images of the boxed area. Top: FUS Middle: RNG105 Bottom: merged. The area surrounded by the dotted line contains the nucleus. Scale bar: 2 μm.

II. Dendritic localization of mRNAs for Arf GEFs and GAPs that are involved in spine formation and maturation in dendrites

Local protein synthesis plays an important role in synaptic plasticity and memory formation. To achieve local translation in dendrites, specific mRNAs are required to be localized to dendrites. RNG105 is a major RNA-binding protein localized to RNA granules, and its deficiency in mice leads to the impairment of higher-order brain functions such as long-term memory and sociability. We previously found that many dendritic mRNAs are reduced in the dendritic layer of the hippocampus in RNG105-deficient mice, particularly mRNAs for ADP-ribosylation factor (Arf) regulators, Arf GEFs and Arf GAPs.

First, we aimed to visualize and quantify the dendritic localization of 8 kinds of mRNAs for the Arf regulators using the MS2 system in mouse cerebrum primary cultured neurons. We found that Arf GEF mRNAs and GAP mRNAs were localized to dendrites in a different way; most of the Arf GEF mRNAs were localized to dendrites independently on KCl stimulation (neuronal depolarization). Despite this, most of the Arf GAP mRNAs were localized to dendrites in a KCl stimulation-dependent manner. Next, we analyzed the knockdown effects of the Arf regulators on the formation of dendritic postsynapses (spines) in primary cultured neurons. Knockdown experiments with shRNA demonstrated that the Arf GEFs and GAPs were classified into two groups: one group consisted of positive regulators of spine formation and maturation, and the other consisted of negative regulators of immature spine formation. These results suggested that Arf GEFs and GAPs both play important roles in tuning spine formation and maturation through their mRNAs being localized to dendrites at a specific timing.

III. Comprehensive behavioral analysis of mice that lack the intrinsically disordered region (IDR) of NFAR2, a stress responsive translation regulatory factor

RNA-binding proteins possessing IDRs, which do not form three-dimensional structures, have been revealed to play an important role in RNA-protein complex assembly and translation regulation. We focused on an RNA-binding protein, NFAR2, that possesses an IDR. NFAR1 and NFAR2 are splicing variants transcribed from a single *ILF3* gene, and inhibit translation of their binding mRNAs in a stress-dependent (*i.e.* oxidative stress) manner. However, they differ in that only NFAR2, but not NFAR1, has the IDR and can associate with RNA granules. To investigate the physiological relevance of the IDR of NFAR2, we generated NFAR2ΔIDR mice, in which a stop codon was introduced in the exon encoding the IDR in the *ILF3* gene.

Comprehensive behavioral analysis demonstrated that NFAR2ΔIDR mice displayed phenotypes that displayed weight loss, body temperature elevation, hyperactivity, decreased anxiety-like behavior, and decreased startle response. Regarding learning and memory, NFAR2ΔIDR mice displayed a decrease specifically in fear-conditioned learning, but not in spatial learning. Furthermore, chronic stress, which is known to induce oxidative stress in the brain, exacerbated the fear-conditioned learning of NFAR2ΔIDR mice without affecting their spatial learning. These results suggested that the IDR of NFAR2 is responsible for specific higher-order brain functions such as fear-conditioned learning under stress conditions.

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 cell malfunction. Our group is interested in the strategies that cells use to protect themselves from alterations in the genome. To date, much information has been 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 it's developmental stage. Our focus is on the genome maintenance mechanisms of 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 of the cell types that compose our body (*i.e.*, ES cells are "pluripotent"). Pluripotent cells exist only transiently and are lost as development proceeds. However, ES cells are capable of proliferating indefinitely when given an appropriate culturing condition. Curiously, ES cells proliferate with truncated gap phases while lengths of S (DNA replication) and M (mitosis) phases are similar to other cell types. ES cells also appear to lack some of the mechanisms that ensure genome integrity (*i.e.*, checkpoint mechanisms). The significance of these phenomena remains a mystery.

To date, studies on cell-cycle regulation in ES cells have not been as 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 has 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 to the extent 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 are taking 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, and is 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, thus allowing us to monitor the initial events of reprogramming after induction.

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).

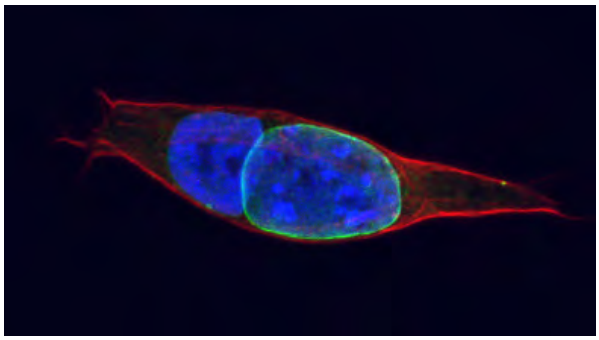

Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus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

III. Future perspectives

While the fundamental mechanisms that maintain genome integrity have been widely studied using various models, the danger a cell might face when altering it's 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.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

LABORATORY OF ORGANELLE REGULATION

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Because plants spread their roots in the ground, they must survive in a given environment. To adapt to their environment, they recognize environmental changes as important signals that are necessary for their survival. In such cases, plant cells can induce, degenerate and differentiate their organelles. The flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aims of this laboratory are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, especially peroxisomes and oil bodies, 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 are involved in various biological processes such as lipid metabolism and photorespiration. These functions are dramatically changed in developmental stages and when confronted with environmental changes. For example, light induces transformation of peroxisomes from glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via β-oxidation and the glyoxylate cycle, to another type of peroxisome, leaf peroxisomes, that function in several crucial steps of photorespiration. After the functional transformation of glyoxysomes to leaf peroxisomes during the greening of cotyledons, the reverse transformation of leaf peroxisomes to glyoxysomes occurs during senescence. Gene expression, alternative splicing, protein translocation, protein degradation and degradation of peroxisomes themselves control these functional transformations.

To better understand peroxisome biogenesis and functions, we isolated a number of Arabidopsis mutants that displayed aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual positioning (*peup* mutants) based on them having a different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes, numbers and distribution could be visualized with GFP.

As of writing, we have reported the function of APEM1, APEM2, APEM3, APEM4, APEM9 and APEM10 (Figure 1). Based on the results we were able to update the model for functional transformation of peroxisomes using these *apem* mutants in concert with the analyses of *peup1*, *peup2* and *peup4* mutants, which were defective in Autophagy-related 2 (ATG2), ATG18a and ATG7, respectively.

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

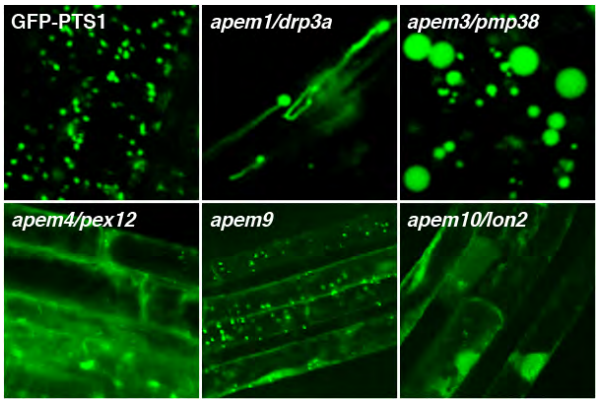

Figure 1. Phenotype of Arabidopsis *apem* mutants. GFP fluorescence was observed in the parent plant, GFP-PTS1, *apem1*, *apem3*, *apem4*, *apem9* and *apem10* mutants. *apem1* and *apem3* have elongated and enlarged peroxisomes, respectively. In *apem4*, *apem9* and *apem10*, GFP fluorescence is observed in the cytosol because of the decrease of the efficiency of protein transport to peroxisomes.

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 in food and industrial materials. Storage reserves vary among different types of plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybeans contain proteins as a major reserve. Storage oils and storage proteins are synthesized in the endoplasmic reticulum (ER) and accumulated in oil bodies and protein bodies, respectively, during the same period of seed development (Figure 2).

We are analyzing the molecular mechanisms controlling oil and protein contents in seeds. Based on the analysis of the temporal sequence of oil and protein synthesis during seed development in *Arabidopsis thaliana*, which produces seeds containing approximately 30% oil and 30% protein, we

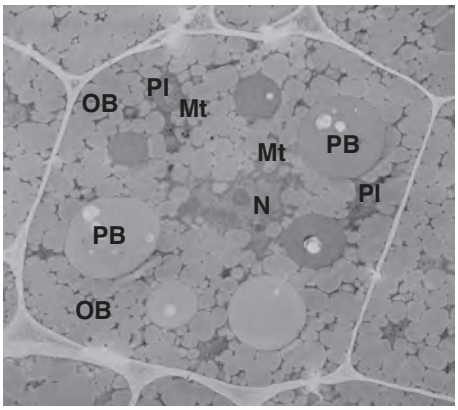

Figure 2. Electron micrograph of Arabidopsis dry seed. Mt, mitochondrion; N, nucleus; OB, oil body; PB, protein body; Pl, plastid.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

revealed that the extension of *WRINKLED1* (*WRI1*), a transcription factor in fatty acid biosynthesis, expression during the midphase of seed development significantly enhanced seed oil content, and caused an enlargement of seed size.

We are also currently investigating the mechanisms of oil accumulation in other plant species. In addition, we are trying to apply our knowledge and techniques to increase beneficial storage reserves.

III. Development of Gateway-technology vectors for plant research

Gateway cloning is a popular technology, which allows the simultaneous generation of multiple constructs containing a range of fusion genes. We have developed various types of Gateway cloning-compatible vectors for the improvement of resources in the plant research field. As of writing, we have provided vector sets to detect multiple protein-protein interactions in vivo using multi-color bimolecular fluorescence complementation, and the binary vectors to facilitate tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha* (Figure 3). We will continue developing other useful Gateway cloning-compatible vectors to contribute to the plant research community.

IV. Construction of The Plant Organelles Database 3 (PODB3) and Plant Organelles World

The Plant Organelles Database 3 (PODB3) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. 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. The function of each database is as follows:

- The electron micrograph database provides information on the ultrastructures in plant cells.
- The perceptive organelles database shows organelles dynamics responding to environmental stimuli.
- The organelles movie database contains time-lapse images and 3D structure rotations.
- The organellome database is a compilation of static image data of various tissues of several plant species at different developmental stages.
- The functional analysis database is a collection of protocols for plant organelle research.

Through these databases, users can easily grasp plant organelle dynamics. Plant Organelles World, which is built based on PODB3, is an educational tool for engaging members of the non-scientific community to explore plant biology. We hope that both PODB3 and Plant Organelles World are of help to researchers as well as the general public.

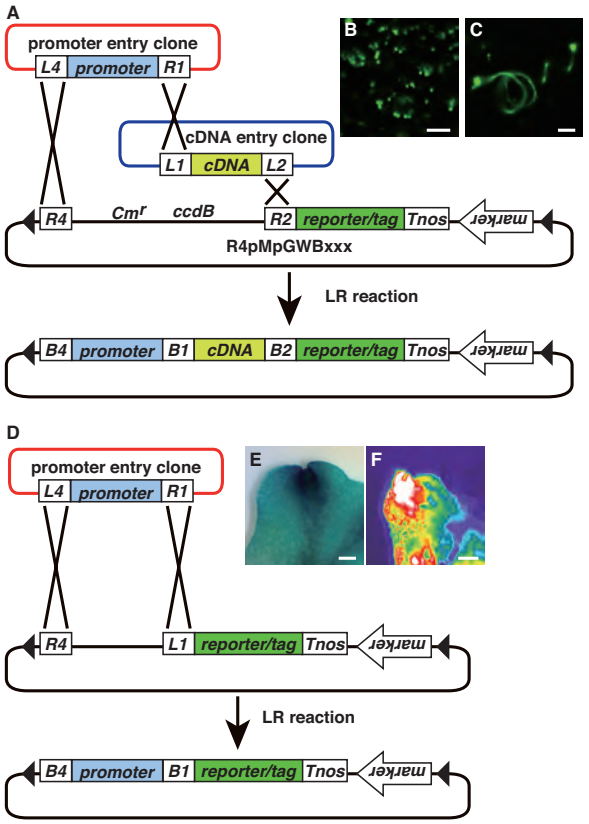

Figure 3. R4pMpGWB and R4L1pMpGWB systems for construction of fusion genes in *M. polymorpha*. (A) In the R4pMpGWB system, promoter and cDNA entry clones and R4pMpGWB vectors are used in a tripartite LR reaction to form a C-terminal fusion of cDNA-encoded protein and a reporter or tag. (B) Thallus epidermal cells expressing the peroxisome-targeted Citrine under the 35S promoter. (C) Sperm cell expressing Lifeact-Venus under the endogenous *PROTAMINE* promoter Bars: 10 μm for (B) and 2 μm for (C). (D) In the R4L1pMpGWB system, the promoter entry clones and R4L1pMpGWB vectors are used for a bipartite LR reaction. (E) GUS staining in thalli expressing *pro*Mp*EF1α:GUS*. (F) Luminescence images after heat shock of transgenic plants bearing *pro*Mp*HSP17.8A1:ELuc(PEST)*. Bars: 1 mm

Publication List:

〔Original paper〕

- Fujikawa, Y., Suekawa, M., Endo, S., Fukami, Y., Mano, S., Nishimura, M., and Esaka, M (2018). Effect of mutation of C-terminal and heme binding region of Arabidopsis catalase on the import to peroxisomes. Biosci. Biotechnol. Biochem. *83*. 322-325.
- Mano, S., Nishihama, R., Ishida, S., Hikino, K., Konodo, M., Nishimura, M., Yamato, T.K., Kohchi, K., and Nakagawa, T. (2018). Novel gateway binary vectors for rapid tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha*. PLoS ONE, *13*, e0204964.

〔Review article〕

- Goto-Yamada, S., Hikino, K., Nishimura, M., Nakagawa, T., and Mano, S. (2018). Bimolecular fluorescence complementation with improved gateway-compatible vectors to visualize protein–protein interactions in plant cells. In Methods in Molecular Biology. Two-Hybrid Systems. Methods and Protocol. Oñate-Sánchez, L., ed. (U.S.A., Springer), pp. 245-258.

DIVISION OF MORPHOGENESIS

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The complex morphogenesis of organisms is achieved by the dynamic rearrangement of tissues during embryogenesis in which changes 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 known as “cell polarity”, which confers them asymmetry, is essential. We have been studying how cell polarity is established within cells and how cell polarity is interpreted by cells to apply this information to their behaviors. We attempt to understand the mechanisms underlying these events using several model animals, including frogs, fish, and ascidians. In addition to genes and proteins that are widely known to govern biological phenomena and have been extensively studied in the past several decades, a growing body of evidence now suggests that physical environments that include light, temperature, and internal and external forces influence cellular as well as organismal behaviors. In particular, we are currently investigating the contribution of physical forces using frog embryos and the light-dependent behaviors using coral planula larvae to understand the impact of physical environments on organisms.

I. Mechanical stimulation induces phosphorylation-dependent signaling in *Xenopus* embryos

Cells sense and respond to not only molecular factors, but also mechanical forces, which play various important roles in biological events, such as tissue homeostasis, differentiation and cell migration. 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. The aim of this project is to elucidate the mechanisms of sensing and responding to mechanical stimuli in *Xenopus* embryos.

It is assumed that protein phosphorylation, one of the immediate responses of cells to external stimuli, is dynamically regulated upon the detection of mechanical force. Therefore, we used quantitative phosphoproteome analyses to investigate mechanosensing pathway in *Xenopus laevis* embryos. The mechanical force administered to embryos was applied by centrifugation, which resulted in flattening embryos and expanding tissues. Protein samples from embryos exposed to mechanical forces were analyzed by quantitative mass spectrometry. We detected over 3,500 proteins in each whole proteome sample, and over 8,500 phosphorylated peptides in each sample. Enrichment analyses based on information theory led us to identify the enrichment in cell adhesion molecules, such as focal adhesion and tight junction components within the upregulated phosphorylation sites during force stimulation (Figure 1). In the phosphoproteome analyses, we also identified several protein kinases contributing to these phosphorylation. Additionally, we found that centrifugal force induced a mesenchymal-epithelial transition (MET)-like phenotype in the enrichment analysis based on proteome level.

We also applied mechanical force to embryos via the use of compression generated by a cover glass. The embryos were reduced by approximately 50 % in height, which is associated with flattening and tissue expansion. The mass spectrometry analysis demonstrated that these compressed embryos also showed a similar protein phosphorylation profile similar to centrifuged embryos. This result suggests that tissue expansion induced by these forces may lead to the change in protein phosphorylation as a response to mechanical forces. This is the overall first study of phosphoproteome analyses in *Xenopus laevis* embryos during mechanical sensing and provides mechanistic insights into mechanical force sensing pathways.

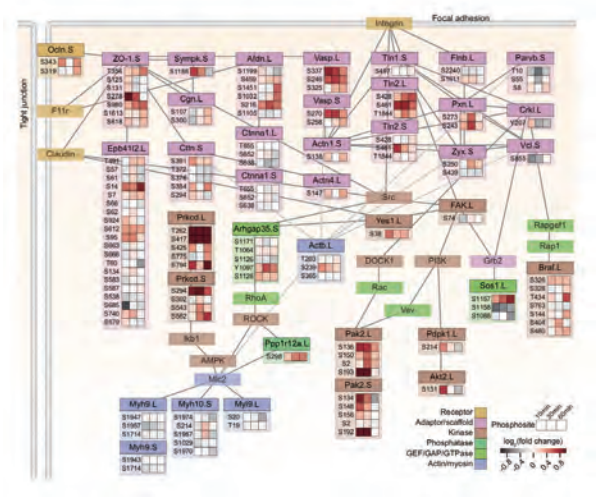

Figure 1. Phosphorylation changes of tight junction and focal adhesion components upon force stimulation in *Xenopus laevis* embryos.

II. Mechanical force regulates tissue stiffness and integrity through FGFR/Erk2 signaling pathway in *Xenopus* embryos

The kinase substrate enrichment analysis based on the quantitative phosphoproteome data demonstrated that the mechanical force applied to *Xenopus* embryos activates

several protein kinases. We focused on one of these protein kinases: a MAP kinase Erk2. We first confirmed that Erk2 is activated similarly in response to both compression and centrifugal force using antibodies against phosphorylated Erk2 (pErk2). As shown in Figure 2, pErk2 was detected within a few minutes after mechanical force applications.

Moreover, we demonstrated that the activated Erk2 regulates integrity and stiffness of the embryonic epithelial cells. The centrifugal force to embryos accumulates apical cortical F-actin layer. In addition to F-actin, the tight junctional protein ZO-1 and the cell adhesion molecule cadherin were accumulated to the apical junctional region. Furthermore. the centrifugal force increased epithelial tissue stiffness of the embryonic surface cells, which was measured by atomic force microscopy (AFM). Importantly, all these cellular changes were found to be dependent on Erk2 activity.

We further examined how Erk2 was activated by this force, and found that inhibition of the FGF receptor (FGFR) diminished Erk2 activation (Figure 2). These observations demonstrated that mechanical force activates FGFR/Erk2 signaling pathway and promotes the formation of mature epithelial junctional structures, which is an indication of MET as predicted by the phosphoproteome analysis described above.

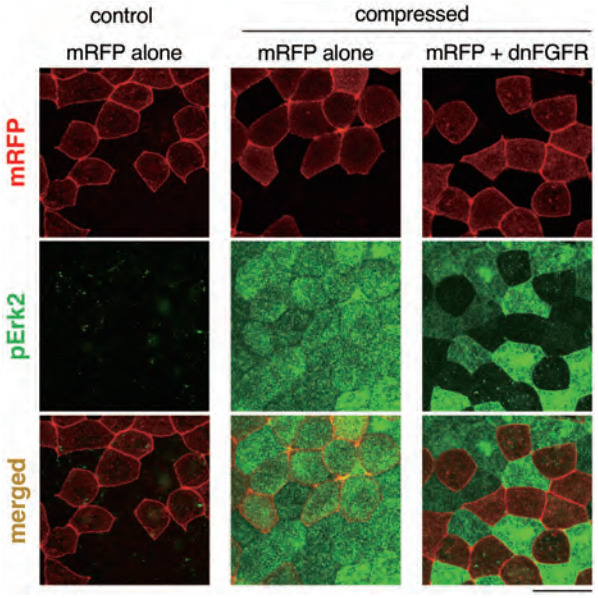

Figure 2. Erk2 is activated by a mechanical force (compression) in *Xenopus* embryonic cells in an FGFR-dependent manner.
mRNA encoding membrane RFP (mRFP) was injected with or without dominant negative FGFR (dnFGFR) mRNA, followed by compression for 5 minutes at the gastrula stage. Embryos were stained with anti pErk2 antibody (green). Superficial ectodermal cells were observed. Bar = 50 μm

III. Mechanical regulation of closing movement and morphology of *Xenopus* neural tube

Neural tube closure (NTC) is an important morphogenetic process during which an anlage of the central nervous system is formed from a flat epithelial sheet called a neural plate (NP). NTC involves tissue-autonomous and non-tissue-autonomous cellular movements that must generate physical forces and modulate mechanical properties of the NP, but how developmental changes of those mechanical forces and properties are integrated for NT morphogenesis is poorly understood. We previously constructed a mathematical model explaining the mechanical regulation of NTC and proposed that the surface elasticities of the neural plate and surrounding tissues affect NTC. In order to examine this hypothesis, we developed an AFM system suitable for measuring mechanical properties of tissues and their temporal changes in developing *Xenopus* embryos. We found that the surface elasticity of the apical side of the NP increased during NTC and showed gradient along mediolateral axis. The substantial stiffening of the apical surface was not observed in the non-neural ectoderm, and required F-actin and non-muscle myosin II, suggesting that the increase of NT-specific actomyosin contractility underlies this stiffening. We also measured the surface elasticity of the basal side of the NT and surrounding mesodermal tissues, and confirmed the highest elasticity measurable on the apical side of the NP. The mechanical properties revealed here should change over time and space ranging from intracellular to tissue levels, and contribute to the large-scale morphogenetic movement of the NP.

IV. Cephalochordate *Brachyury* enhancers involved in evolution of the chordate notochord and somites

The notochord and somite are the most prominent organs in chordates, and *Brachyury* (*Bra*) plays a pivotal role in their formation. The question how *Bra* gained it's enhancer activity in organ-specific expression is therefore critical to our understanding of chordate evolution. In cephalochordate *Bra* is duplicated into two genes (Figure 3). We examined the enhancer activity of *Branchiostoma floridae Bra* (*BfBra1* and *BfBra2*) by *lacZ* reporter assay using a *Ciona*-embryo host system. In the 5', 3' regions and in introns, the signal intensity was higher and broader in *BfBra2* than *BfBra1* (Figure 3). In some cases *lacZ* expression is expanded to posterior muscle cells in the former. The intron enhancers displayed another difference: primary expression of *BfBra1* occurs in notochord whereas both muscle and notochord manifest the expression of *BfBra2*. When considered along with other data, we prefer an EvoDevo scenario in which *BfBra2* is more ancestral and/or mother-like and *BfBra1* is daughter-like and duplicated by BfBra1. Originally, ancestral *BfBra2* is likely to gain enhancer machinery for gene expression in muscle and notochord, as has been deduced from its spatial expression profile: the 5' region for somite expression, the 3' region for notochord expression, and introns for somite/notochord expression. In contrast, sister *BfBra1* became more specialized for notochord expression using the intron enhancers.

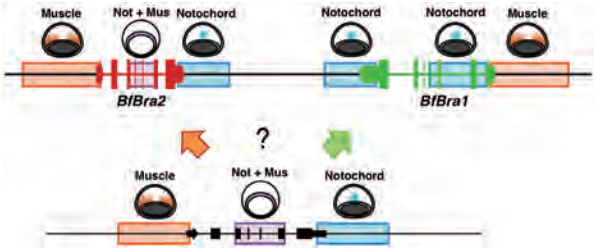

Figure 3. A summary of results of the present reporter assay and possible interpretation.
Enhancer activity of 5' upstream sequences in muscle is shown in brown and that of the 3' downstream in notochord is in blue. The activity *BfBra2* introns in muscle and notochord is shown in purple.

V. Light-dependent swimming behavior of coral larvae of *Acropora tenuis*

Many reef-building corals form a symbiotic relationship with dinoflagellate algae of the genus *Symbiodinium*. Corals mostly depend on photosynthetic products from these symbionts as 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 a lack of basic photobiological studies in corals, how coral larvae perceive and respond to light environment remain largely unknown. To answer these questions, we analysed the effects of light stimuli on larval swimming behavior. In addition, by focusing on opsin-like photopigments, we have been examining the mechanism of photoreception in a reef-building coral, *Acropora tenuis* (Figure 4).

First, we precisely observed the larval swimming activity under fluctuating light conditions, and found that larvae temporarily stopped swimming ~30 s after the rapid reduction of light intensity. This behavior was also observed when we rapidly changed spectral composition ("color") of light. Further experiments using the Okazaki Large Spectograph revealed that the loss of short wavelengths of light (blue/green light) induced this type of behavior. This light-dependent behavior might lead to larval aggregation to optimal light environments in natural habitat.

As for the analysis of photoreception, we identified eight opsin-like genes on the *A. tenuis* genome and measured the absorbance spectra of one of these eight opsins (s0350 opsin). We found this opsin absorbed UV light and UV dependent activation of s0350 opsin caused the elevation of intracellular cAMP level. This indicates that s0350 opsin functions as a UV-sensitive photorecetor. Therefore, *A. tenuis* larvae sense environmental UV light and the information they attain may play an important role in their behavior and settlement.

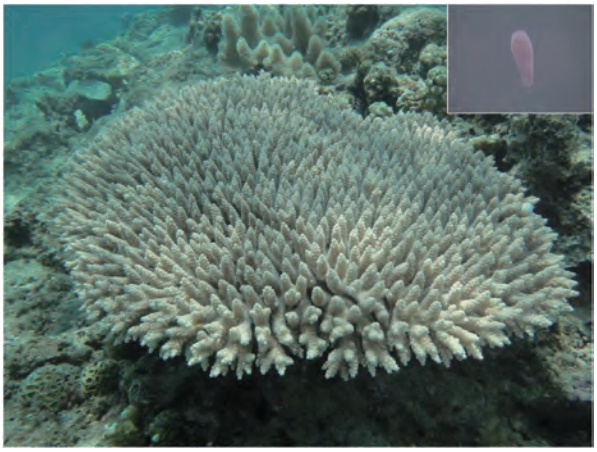

Figure 4. A wild colony of *Acropora tenuis* (photo taken by Masayuki Hatta at Ochanomizu University). The inset shows a planula larvae of *A. tenuis*.

Publication List:

〔Original papers〕

- Harris, A., Siggers, P., Corrochano, S., Warr, N., Sagar, D., Grimes, D.T., Suzuki, M., Burdine, R.D., Cong, F., Koo, B.K., Clevers, H., Stévant, I., Nef, S., Wells, S., Brauner, R., Ben Rhouma, B., Belguith, N., Eozenou, C., Bignon-Topalovic, J., Bashamboo, A., McElreavey, K., and Greenfield, A. (2018). ZNRF3 functions in mammalian sex determination by inhibiting canonical WNT signaling. Proc. Natl. Acad. Sci. USA *115*, 5474-5479.
- Hayashi, K., Yamamoto, T.S., and Ueno, N. (2018). Intracellular calcium signal at the leading edge regulates mesodermal sheet migration during *Xenopus* gastrulation. Sci. Reports *8*, 2433.
- Jean Beltran, P.M., Cook, K.C., Hashimoto, Y., Galitzine, C., Murray, L.A., Vitek, O., and Cristea, I.M. (2018). Infection-induced peroxisome biogenesis is a metabolic strategy for herpesvirus replication. Cell Host Microbe, *24*, 526-541.
- Shinoda, T., Nagasaka, A., Inoue, Y., Higuchi, R., Minami, Y., Kato, K., Suzuki, M., Kondo, T., Kawaue, T., Saito, K., Ueno, N., Fukazawa, Y., Nagayama, M., Miura, T., Adachi, T., and Miyata, T. (2018). Elasticity-based boosting of neuroepithelial nucleokinesis via indirect energy transfer from mother to daughter. PLoS Biol. *16*, e2004426.
- Tominaga, H., Satoh, N., Ueno, N., and Takahashi, H. (2018). Enhancer activities of amphioxus *Brachyury* genes in embryos of the ascidian, *Ciona intestinalis*. Genesis *56*, e23240.

〔Review article〕

- Yokoyama, H., Kudo, N., Todate, M., Shimada, Y., Suzuki, M., and Tamura, K. (2018). Skin regeneration of amphibians: A novel model for skin regeneration as adults. Dev. Growth Differ. *60*, 316-325.

DIVISION OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

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

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

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

I. Structural, biophysical and biochemical characteristics of secreted Wnt proteins

The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis. It has been traditionally 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 the situation. In accordance with this line of thinking, various models have been proposed for Wnt transport between producing and receiving cells (Figure 1). Thus, to better understand the spatial regulation of tissue morphogenesis, the molecular mechanism underlying the spreading of Wnt proteins should be revealed.

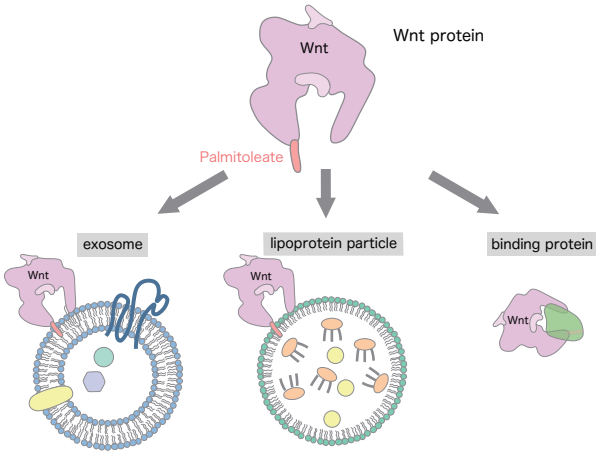

Figure 1. Various extracellular deliverers associating with Wnt involved in Wnt trafficking; extracellular membranous deliverers, including exosomes and lipoprotein particles, are known to associate with Wnts. In addition, specific Wnt-binding proteins, including Swim in Drosophila and sFRPs in vertebrates, were reported to be involved in extracellular Wnt trafficking. These various machineries appear to modulate the Wnt signaling range in differing ways.

To attain a better understanding of 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 non-invasive methods for this characterization. We established an L cell line, in which mouse Wnt3a tagged with monomeric GFP was efficiently produced, and analyzed the size of GFP-tagged Wnt3a secreted in the conditioned medium. The average size of GFP-Wnt3a, which was estimated by measuring the diffusion constant of fluorescent particles using Fluorescence Correlation Spectrometry (FCS), was larger than that predicted for the monomer.

To further examine Wnt proteins in the presence of serum, we used analytical ultracentrifugation in combination with a fluorescence detection system (AUC-FDS), which allowed us to monitor sedimentation of GFP-tagged proteins. While

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The formertitle is indicated by an asterisk (*).

no peak corresponding to the monomeric form of Wnt3a was detected, a major peak with the apparent molecular mass of ~150 kDa was observed. Biochemical analysis including immune-depletion revealed that this peak corresponded to a complex with a serum protein called afamin, which was recently reported to bind with Wnt proteins in the culture medium.

In addition, AUC-FDS analysis revealed that secreted Wnt3a form high-molecular weight (HMW) complexes at their smallest peak with an apparent molecular mass of ~200 kDa. We further examined the HMW complexes more precisely by using FLAG-tagged Wnt3a, which can reduce the steric effect of tagged peptides than GFP-tagged ones. After affinity purification with anti-FLAG antibodies, purified Wnt3a proteins were separated by gel filtration. Western blotting of each fraction using Blue Native PAGE, which can separate protein complexes maintaining native conformation, indicated that the size of Wnt3a protein complex is gradually distributed between ~150 kDa and 1200 kDa.

Interestingly, the smallest fraction of Wnt3a proteins exhibited a distinct band in Blue Native PAGE analysis. Since Wnt3a is the dominant protein in this fraction, this result strongly suggested that Wnt3a forms a discrete homo-oligomer. Judging from the size of this distinct band, this oligomer corresponds with a trimer. Cross-linking analysis with this fraction confirmed as much. These findings are in agreement with the AUC-FDS results, where the apparent molecular mass of the smallest HMW complex was similar to that of GFP-Wnt3a homo-trimer. All of this evidence indicates that homo-trimers are the smallest form of the HMW complexes. Furthermore, crosslinking analyses with other Wnt3a containing fractions suggested that the homo-trimer is the smallest structural unit of larger HMW complexes recovered from other fractions.

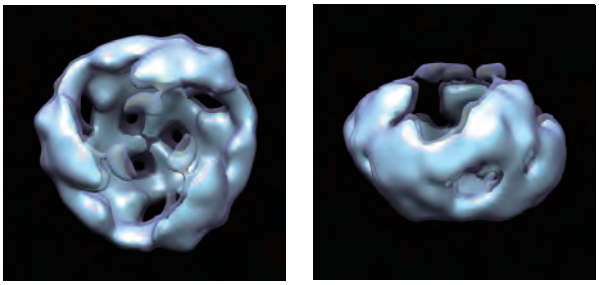

Figure 2. Image of Wnt3a reconstructed by single particle analysis. Top view (right) and side view (left) are shown.

Electron microscopic images of protein particles in each fraction revealed that the size and shape of most particles in the smallest fraction looked relatively uniform. We attempted to reconstruct a 3D structure of Wnt trimer by single particle analysis (Figure 2). As expected, the reconstructed image shows a three-fold rotational symmetry structure, indicating that three Wnt3a molecules were arranged on the same circumference of the circle in order to overlap each other. Given that the palmitoleoylate adduct should be shielded from aquatic environments, the partial overlapping would be beneficial in this case.

II. Regulation of spatial distribution of Wnt proteins in vertebrate embryos

To examine the mobility of Wnt3a in the extracellular milieu, we performed FCS analysis of GFP-Wnt3a in *Xenopus* embryos. This examination revealed that the dynamic behavior of Wnt3a could be divided into two distinct states. The fast population appears to reflect quickly and freely diffusing molecules, and the slow population reflects slowly moving molecules probably by interacting with extracellular matrices. Further precise analyses revealed that HMW complexes were less mobile than relatively smaller ones.

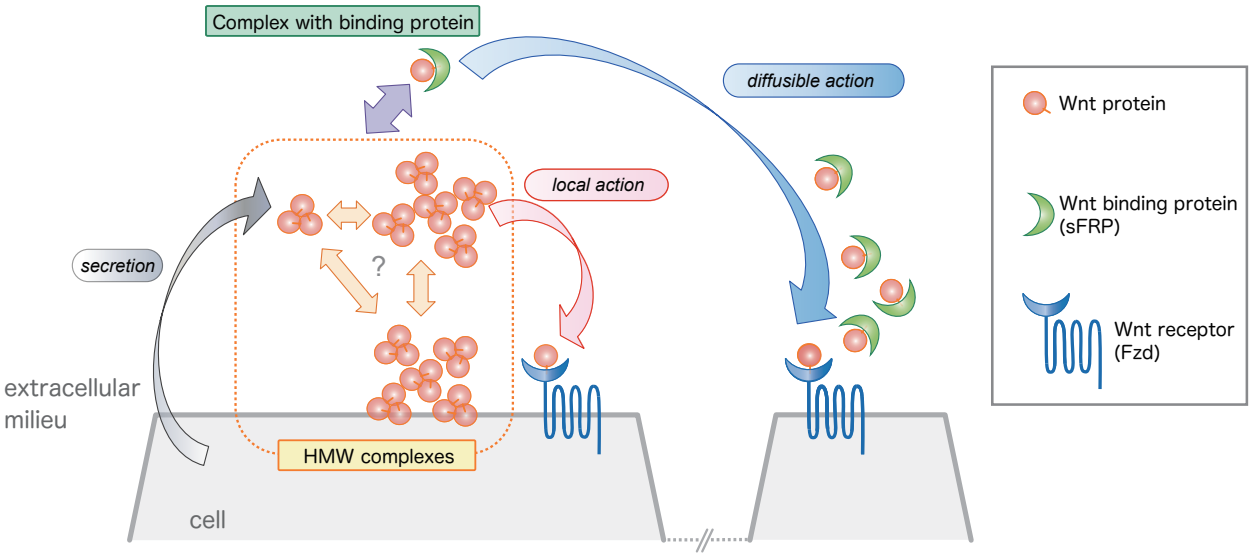

Figure 3. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu although it is uncertain when the assembly to the HMW complex occurs during the process of Wnt secretion. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion range. Some Wnt molecules can be dissociated by local interaction with Frizzled receptor (Fzd), resulting in a short-range signal (local action). In contrast, the HMW complex, probably as well as the trimer itself, can also be dissociated by interaction with soluble Wnt binding protein (partner protein), including sFRP. By this dissociation, Wnt turns to be more mobile and its diffusion range is expanded (diffusible action).

We showed that the trimer and larger HMW complexes of Wnt3a proteins could be dissociated by interaction with their receptor Frizzled8 and with secreted Wnt binding protein, sFRP2, in vitro by utilizing AUC-FDS. Similarly, this dissociation was detected in vivo by FCS. Importantly, the results of FCS suggested that dissociation of the large assembly of Wnt3a proteins by interaction with sFRP can make Wnt more mobile, probably resulting in longer diffusion distance in the embryo. These ideas are supported by a finding that states that the distribution range of Wnt3a was expanded by the co-expression of sFRP2 in *Xenopus* embryos. These results showed that large assemblies of Wnt3a are less mobile and sFRP2 can expand the diffusion range of Wnt proteins in *Xenopus* embryos. Based on these results, we propose a model that contends that assembly and dissociation of the dissociable oligomers modulate Wnt signaling range (Figure 3).

III. 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 at the posterior of 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 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 them. This spatial pattern is established in the anterior PSM by converting the oscillatory gene expression into spatially restricted patterns of gene expression. Several intracellular 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 this and other groups strongly suggest that Mesp2 does not directly define the position of the segmentation boundary, rather that 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.

Publication List:

〔**Original papers**〕

- Kinoshita, H., Ohgane, N., Fujino, Y., Yabe, T., Ovara, H., Yokota, D., Izuka, A., Kage, D., Yamasu, K., Takada, S., and Kawamura, A. (2018). Functional roles of the Ripply-mediated suppression of segmentation gene expression at the anterior presomitic mesoderm in zebrafish. Mech. Dev. *152*, 21-31.
- Takada, R., Mii, Y., Krayukhina, E., Maruyama, Y., Mio, K., Sasaki, Y., Shinkawa, T., Pack, C.-G., Sako, Y., Sato, C., Uchiyama, S., and Takada, S. (2018). Assembly of protein complexes restricts diffusion of Wnt3a proteins Commun. Biol. *1*, 165.
- Tsuchiya, Y., Mii, Y., Okada, K., Furuse, M., Okubo, T., and Takada, S. (2018). Ripply3 is required for the maintenance of epithelial sheets in the morphogenesis of pharyngeal pouches Dev. Growth Diff. *60*, 87-96.

Division of Embryology

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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 the interaction between the embryo and maternal tissue is essential in this process. Another characteristic is the highly regulative potential of embryos. Cells within embryos interact with their environment and establish their own fates and behaviors. We want to provide basic and fundamental information about the specification of the differentiation of cell lineages, embryonic axes, cell behaviors, body shape regulation 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, even mammalian ones, in real time. 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 across many laboratories over the world. We have also established mouse lines to monitor the cell cycle. We have been studying the behavior of cells within embryos by applying newly developed image analyses following the observation of cell behaviors within embryos using these mouse lines. We found that the proximal visceral endoderm overlying the extra-embryonic ectoderm shows coherent cell growth in a proximal-anterior to distal-posterior direction. We also observed that directional cell migration is coupled with cell elongation in the anterior region, suggesting that the behaviors of visceral endoderm cells vary between regions during peri-implantation stages.

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 the behaviors of cells and 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 the developmental biology of mammalian embryos, embryos are usually removed from the uterus, and those that are isolated are analyzed. We have been analyzing the early embryonic development of the mouse by 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 it 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 the molecules involved in the interaction between the embryo proper and uterine cells, which may play a major role in embryonic development.

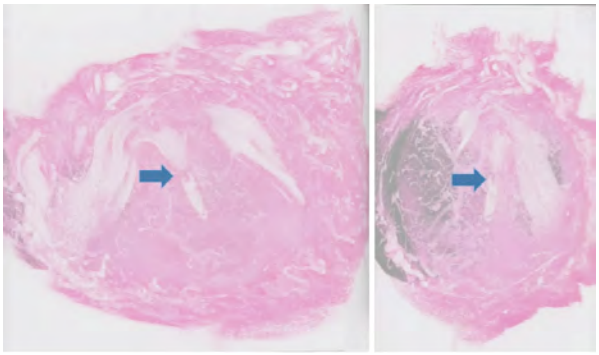

Figure 1. Three-dimensional image reconstruction of the pregnant uterus. A uterus obtained 5 days after fertilization was sectioned and images were digitized by an image scanner, and reconstructed three-dimensionally to observe the relationship between the embryo and the maternal tissue. The right panel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

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The formertitle is indicated by an asterisk (*).

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 multi-cilia 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 a polarized manner 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 of cilia 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 a 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.

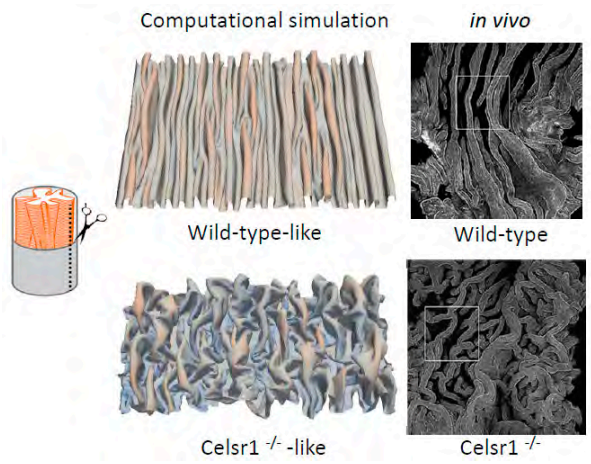

Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

Epithelial folds are observed in various organs including the oviducts, guts, and airways *etc*. Longitudinally aligned folds are also observed in the oviducts of birds and frogs. 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 and branched folds which are observed in wild-type and *Celsr1* mutant mice, respectively (Figure 2). In addition, our model also successfully reproduced circumferentially aligned folds and zigzag folds observed in other organs. We are also focusing on some other PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain their polarity.

IV. Analysis of the 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 statistical 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-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.

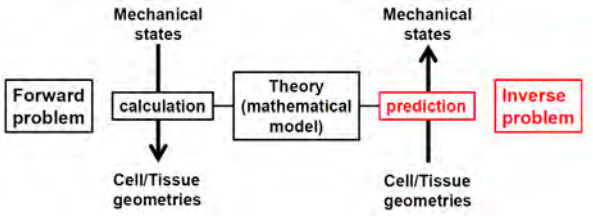

Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

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 the 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 to the cell membrane. Series of data show that PIEZO2 serves as the main mechanosensor in sensory neurons for light touch sen-

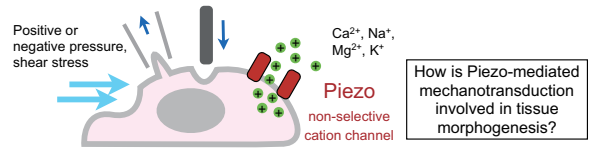

Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

sation, proprioception and breathing. We recently found that PIEZO1 in endothelial cells is required for lymphatic valve formation (Figure 5). To further elucidate how PIEZO1-mediated mechanotransduction is involved in lymphatic valve formation, we have been developing systems to manipulate mechanical stimuli and monitor 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.

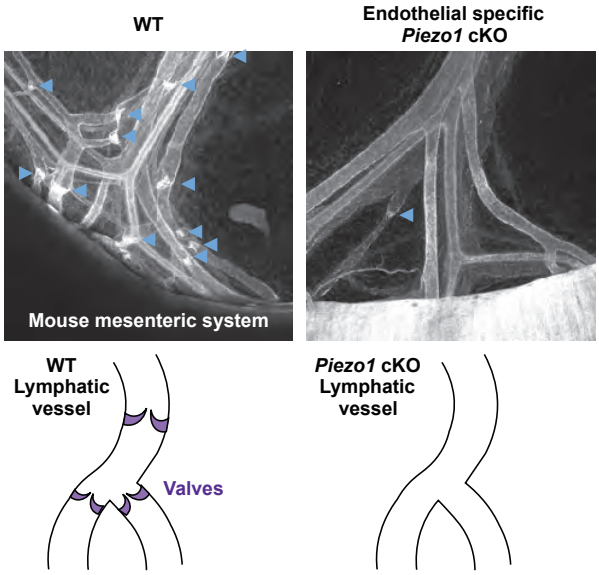

Figure 5. Reduced number of lymphatic valves in mice lacking PIEZO1 in endothelial cells.

VI. Mechanics of cell population patterning during development

During development, cells actively and/or passively move, resulting in various cell distribution patterns. We investigated the effect of passive cell movements provoked by frictional forces from adjacent growing tissues. The passive movements generated various patterns, such as an elongated cell cluster and multiple cell clusters, *etc*. Difference in cellular stiffness was critical for these pattern formations. The former situation is actually observed during elongation of the notochord in mice. Together with active cell movements and the differential adhesion hypothesis which has been recognized as a classical concept within biology, passive cell movements can be effective for diverse pattern formation.

Publication List:

〔Original papers〕

- Abe, T., Kutsuna, N., Kiyonari, H., Furuta, Y., and Fujimori, T. (2018). ROSA26 reporter mouse lines and image analyses reveal the distinct region-specific cell behaviors in the visceral endoderm. Development *145*, dev165852.
- Kikuchi, K., Nakamura, A., Arata, M., Shi, D., Nakagawa, M., Tanaka, T., Uemura, T., Fujimori, T., Kikuchi, A., Uezu, A., Sakamoto, Y., and Nakanishi, H. (2018). Map7/7D1 and Dvl form a feedback loop that facilitates microtubule remodeling and Wnt5a signaling. EMBO Rep. *19*, e45471.
- Nonomura, K., Lukacs, V., Sweet, D.T., Goddard, L.M., Kanie, A., Whitwam, T., Ranade, S.S., Fujimori, T., Kahn, M.L., and Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc. Natl. Acad. Sci. USA *115*, 12817-12822.
- Xu, J., Mathur, J., Vessieres, E., Hammack, S., Nonomura, K., Favre, J., Grimaud, L., Petrus, M., Francisco, A., Li, J., Lee, V., Xiang, F.L., Mainquist, J.K., Cahalan, S.M., Orth, A.P., Walker, J.P., Ma, S., Lukacs, V., Bordone, L., Bandell, M., Laffitte, B., Xu, Y., Chien, S., Henrion, D., and Patapoutian, A. (2018). GPR68 senses flow and is essential for vascualr physiology. Cell *173*, 762-775.

〔Review article〕

- Koyama, H., and Fujimori, T. (2018). Biomechanics of epithelial fold pattern formation in the mouse female reproductive tract. Curr. Opin. Genet. Dev. *51*, 59-66.

Division of Germ Cell Biology

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Overview of our research

Production of numerous sperm over a long period in the testis is fundamental for continuity of life across generations. The Division of Germ Cell Biology aims to understand the key processes of germ cell development that lead to robust sperm formation.

Our emphasis has specifically been on the regulation and functional behavior of spermatogenic stem cells (SSCs) in the testes of mice. SSCs play a crucial role, not only as the origin of long-term sperm production ensuring the continuity of life, but also as the crucible of genetic and potentially epigenetic mutations, the underpinning of evolution as well as congenital disease mechanisms. The principal and signature strategy of our research is to directly *observe* the behavior of individual germ cells *in vivo* in their natural (*i.e.* physiological) state and take advantage of intravital live-imaging, lineage analysis, and biophysical analysis.

Regarding the study of SSCs, first, we found that SSCs include a functional hierarchy, comprised of an "actual" stem cell compartment that is able to self-renew, and a differentiation-primed, "potential" stem cell compartment. We have been also investigating the cellular identity of "actual" SSCs, their *in vivo* behavior at a single-cell resolution, and the underlying mathematical principles, leading to the discovery of the dynamics of "population asymmetry" and subsequent "neutral competition" between SSCs. We are also highly interested in the area of "potential stem cells". In steady-state spermatogenesis, these largely contribute as transient amplifying cells and differentiate rather than self-renew. However, when tissues are insulted or testicular cells are transplanted into host testes, their probability of self-renewal increases and they effectively replenish the lost "actual" stem cells. Such flexible and probabilistic features of stem cells have been found to be paradigmatic for many other stem cell-supported tissues.

Our past key references related to these studies include Nakagawa *et al*., Dev. Cell 2007; Yoshida *et al*., Science 2007; Nakagawa *et al*., Science 2010; Klein *et al*., Cell Stem Cell 2010; Hara *et al*., Cell Stem Cell 2014, Ikami *et al*., Development 2015, and Tokue *et al*., Stem Cell Reports 2017.

Mechanism of "mitogen competition" regulates the stem cell density homeostasis in an "open" niche

In 2018, we developed a novel hypothesis regarding the mechanism of SSC maintenance in the mouse testis (Kitadate *et al*., Cell Stem Cell 2019). In contrast to better-investigated tissues such as *Drosophila* testes and ovaries, as well as mammalian intestinal crypts, where physical contact made by stem cells with an anatomically-defined niche provides a platform to maintain homeostasis, the question of how stem cell homeostasis is achieved in the mouse testis remains unknown. In the mouse testes' seminiferous tubules, the place where spermatogenesis proceeds, SSCs are motile and dispersed among their progeny, thus representing a class of microenvironment designated as an "open niche" (Yoshida, Dev. Growth. Diff. 2008). Intriguingly, the density of SSCs (defined as GFRα1$^+$ cells) is strikingly constant when averaged over a certain length (*e.g.*, >1cm), despite local fluctuations. This suggests a presence of an unknown mechanism that regulates the SSC homeostasis in a manner that stabilizes their average density.

I. FGF5 expression near the vasculature and its mitogenic function on GFRα1$^+$ spermatogonia

In order to find the key factors regulating the GFRα1$^+$ cell density, we screened the genes expressed in the tubule area

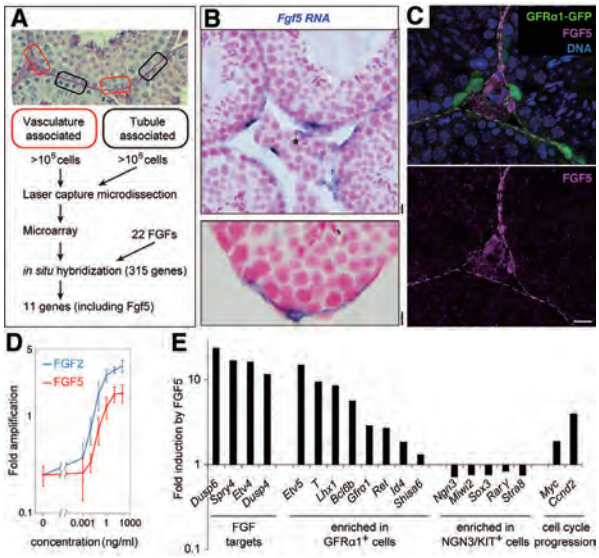

Figure 1. (A) Outline of the screening for genes preferentially expressed in the vasculature-associated region. (B) Representative ISH images for *Fgf5* (blue) in testis sections, counterstained with nuclear fast red. (C) Representative image of an inter-tubular region of a GFRα1-GFP mouse testis stained for GFP (green), FGF5 (magenta) and DNA (blue). Scale bars, 10 μm. (D) Mitogenic effect of FGF5 (red) or FGF2 (blue) on cultured spermatogonia. Fold increase in the number of GS cells cultured with indicated concentration of FGF5 for 8 days. Shown in average ± SEM (n = 3 independent experiments). (E) Effects of FGF5 on gene expression. GS cells depleted of FGF2 and GDNF for 3 days were supplemented with or without FGF5 (100 ng/ml) for 24 hours, followed by cDNA microarray analyses. * intertubular arterioles/venules All figures are reproduced from modifications taken from Kitadate *et al*., Cell Stem Cell (2019).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The formertitle is indicated by an asterisk (*).

that were facing the interstitium and vasculature, where GFRα1⁺ cells preferentially locate (Figure 1A). From this screening, we focused on *Fgf5*, since it is located in peritubularly located large flattened cells (termed lymphatic endothelial cells) near the interstitium (Figures 1B and C). By using an *in vitro* spermatogonial culture, we found that FGF5 showed mitogenic and anti-differentiation effects (Figures 1D and E).

II. FGF5 controls GFRα1⁺ cell density in a linear dosage-dependent manner

We then investigated the *in vivo* role of FGF5 in mice carrying a null allele (*Fgf5*⁻) or an extra copy of BAC-mediated transgene (*BAC-Fgf5*^{Tg}). In mutant testes, the average density of GFRα1⁺ spermatogonia showed a strikingly linear correlation with *Fgf5* dosage (Figure 2A). These mutants notably sustained steady-state spermatogenesis with different density *set points* of GFRα1⁺ cells that correlate in a manner that depends linearly on *Fgf5* dosage during adulthood (Figure 2B).

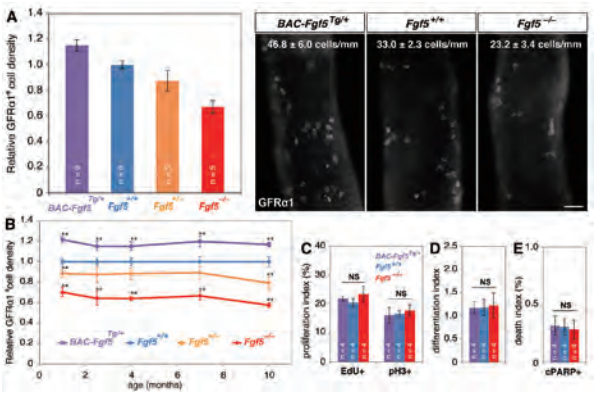

Figure 2. (A) Average densities ± SEM of GFRα1⁺ cells and representative IF images of whole-mount seminiferous tubules for GFRα1 (right) of 2.5 month-old mice with indicated genotypes. Scale bar, 50μm. (B) Average densities ± SEM of GFRα1⁺ cells in mice with indicated genotypes and ages. (C–E) Indexes of proliferation (EdU⁺ and pH3⁺ fractions), differentiation [quantified as the RARγ⁺/KIT⁻ (≈NGN3⁺) over GFRα1⁺ cell ratio], and death (cPARP⁺ fraction) in GFRα1⁺ cells of indicated mice at 2.5 months of age.

III. Each GFRα1⁺ cell receives an unchanged level of FGF signal in *Fgf* mutants

Given the mitogenic and differentiation-inhibiting functions of FGF5, we first considered whether GFRα1⁺ cells receive altered levels of FGF signal in mutants, which in turn changes their fate resulting in altered densities. However, the rates of proliferation, differentiation and death of GFRα1⁺ cells, as well as their gene expression profile, surprisingly were not different between *Fgf5*$^{-/-}$, *BAC-Fgf5*$^{Tg/+}$ and WT mice (Figures 2C–E; not shown), indicating conserved fate behavior of GFRa1⁺ cells between mutants.

The above findings lead to a hypothesis that FGF supply is a limiting factor that is competed for among the GFRα1⁺ cells. In this case, the levels of FGF signal received by each GFRα1⁺ cell would be equalized among *Fgf* mutants harboring different densities of GFRα1⁺ cells.

IV. GFRα1⁺ spermatogonia consume FGF5

To further develop this hypothesis, we then examined whether GFRα1⁺ cells consume the extracellular FGF5 when they receive its signal *in vivo*, as this was probably the simplest form of competition consistent with the general mechanism of FGF signal reception by target cells. We detected speckled FGF5 signals inside GFRα1⁺ cells (Figure 3A). Significant portions of FGF5 cytoplasmic signals were co-localized with SDC4 as cytoplasmic puncta on LAMP1⁺ lysosomes (Figures 3B). These observations indicated that GFRα1⁺ cells consume extracellular FGF5, supporting the idea that GFRα1⁺ cells compete for extracellular FGF.

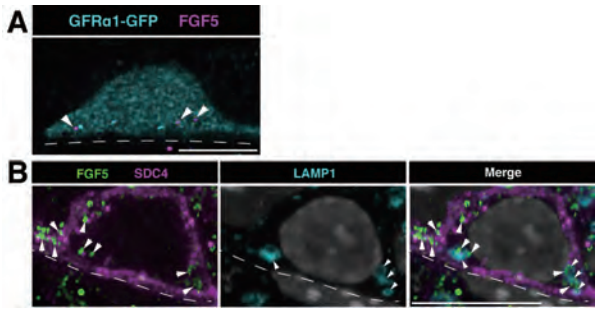

Figure 3. (A) Representative images of GFRα1-GFP⁺ (cyan) cells exhibiting the speckled cytoplasmic staining of FGF5 (magenta, white arrowheads). (B) Representative images of SDC4⁺ (magenta) cells co-stained for FGF5 (green) with LAMP1 (cyan). FGF5⁺ speckles (arrows) were often co-localized with LAMP1⁺ (arrowheads) foci in SDC4⁺ cytoplasmic clamp. Scale bars, 10 μm.

V. Homeostatic stem cell density regulation follows from a model of "mitogen competition"

To understand the mechanism of density regulation, we developed a minimal theoretical model, in which GFRα1⁺ cells are exposed to a steady supply of FGF from the lymphatic endothelial cells, which in turn elicits the concentration-dependent mitogenic and differentiation-inhibiting activities, while also being consumed by, the GFRα1⁺ cells.

This model was found to be capable of capturing the counterintuitive observation that the fate behavior of GFRα1⁺ cells does not change in *Fgf* mutants (Figures 2C–E), as a consequence of the steady-state FGF concentration being always pinned and at which the increase (renewal) and decrease (differentiation) of GFRα1⁺ cells is balanced. Given that GFRα1⁺ cells effectively compete with each other for the limited supply of FGFs, we refer to this mechanism as the "mitogen competition ".

We then questioned whether the model could also predict quantitatively the dynamics of stem cells, if the sytem is strongly perturbed from a steady state. Analysis of the model predicted that the recovery of GFRα1⁺ cell density following a strong perturbation from its steady-state (viz. uninjured) value would show decaying oscillations. To test this prediction, we examined the kinetics during regeneration following the reduction of GFRα1⁺ cells by injecting a cytotoxic reagent, busulfan. Strikingly, decaying oscillations that converged into the steady-state value over several months were indeed observed, with a profile that quantitatively matched with our theory (Figure 4).

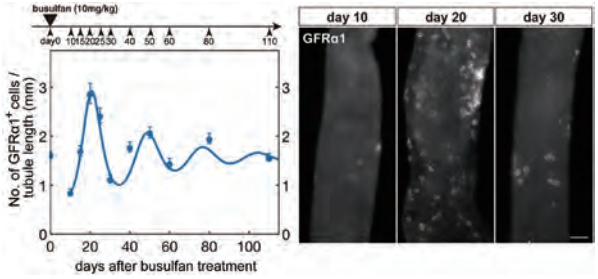

Figure 4. Observed kinetics of the GFRα1⁺ cell density following busulfan treatment in WT (left), and examples of IF images of whole-mount seminiferous tubules stained for GFRα1 (right). Model results (curves) compared to experimental measurements (dots) of the average GFRα1⁺ cell density following busulfan treatment.

To summarize, our results show that the *in vivo* fate behavior of GFRα1⁺ cells is regulated by the mitogenic and anti-differentiation effects of FGFs released in proximity to the vasculature (Figure 5). We propose that competition for mitogens might be a paradigmatic mechanism that can explain the regulation of stem cell homeostasis in a wide range of tissues.

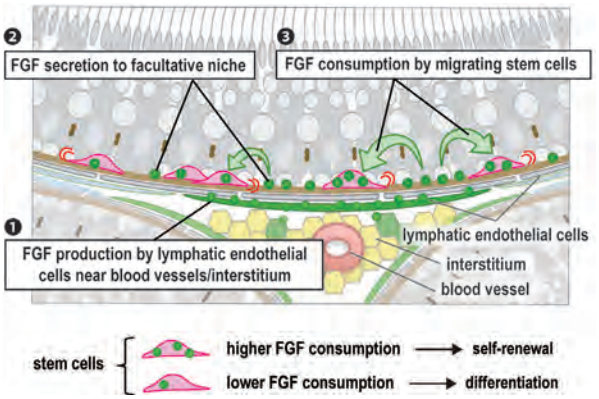

Figure 5. A conceptual diagram of mitogen competition leading to stem cell density homeostasis. Limited supply of FGF from a subset of lymphatic endothelial cells in proximity to vasculature plays a key role in stem cell density homeostasis.

Publication List:

〔Original Papers〕

- Sakamoto, S., Thumkeo, D., Ohta, H., Zhang, Z., Huang, S.R., Kanchenawong, P., Fuu, T., Watanabe, S., Shimada, K., Fujihara, Y., Yoshida, S., Ikawa, M., Watanabe, N., Saitou, M., and Narumiya, S. (2018). mDia1/3 generate cortical F-actin meshwork in Sertoli cells that is continuous with contractile F-actin bundles and indispensable for spermatogenesis and male fertility. PLoS Biol. *16*, e2004874.
- Shawki, H.H., Oishi, H., Usui, T., Kitadate, Y., Basha, W.A., Abdellatif, A.M., Hasegawa, K., Okada, R., Mochida, K., El-Shemy, H.A., Muratani, M., Ogura, A., Yoshida, S., and Takahashi, S. (2018). MAFB is dispensable for the fetal testis morphogenesis and the maintenance of spermatogenesis in adult mice. PLoS ONE *13*. e0190800.

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

- Kitadate, Y., Jörg, D.J., Tokue, M., Maruyama, A., Ichikawa, R., Tsuchiya, S., Segi-Nishida, E., Nakagawa T., Uchida, A., Kimura-Yoshida, C., Mizuno, S., Sugiyama, F., Azami, T., Ema, M., Noda, C., Kobayashi, S., Matsuo, I., Kanai, Y., Nagasawa, T., Sugimoto, Y., Takahashi S., *Simons, B.D., and *Yoshida, S. Competition for mitogens regulates spermatogenic stem cell homeostasis in an open niche. Cell Stem Cell. 2018 Dec 20. (*: Co-corresponding authors)

〔Review Articles〕

- Yoshida, S. (2018). Open niche regulation of mouse spermatogenic stem cells: Develop. Growth Differ. *60,* 542-552.
- Yoshida, S. (2018). Regulatory mechanism of spermatogenic stem cells in mice: their dynamic and context-dependent behavior, In Reproductive and Developmental Strategies, K. Kobayashi, T. Kitano, Y. Iwao, and M, Kondo, eds. (Springer Japan), pp. 47-67.

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The scope of our interests encompasses the 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 learning and memory

Brain-derived neurotrophic factor (BDNF) plays an important role in synaptic plasticity related to learning and memory. While we have previously reported that SPARC-related protein containing immunoglobulin domains 1 (SPIG1, also known as Follistatin-like protein 4, FSTL4) binds to pro-BDNF and negatively regulates BDNF maturation, its neurological functions, particularly in learning and memory, have not yet been elucidated.

To this end, we examined the electrophysiological and behavioral phenotypes of *Spig1*-knockout (*Spig1*-KO) mice. Adult *Spig1*-KO mice exhibited greater excitability and facilitated long-term potentiation (LTP) in the CA1 region of hippocampal slices than age- and sex-matched wild-type (WT) mice (Figure 1A). Facilitated LTP was reduced to the level of WT by the bath application of an anti-BDNF antibody to hippocampal slices. A step-through inhibitory avoidance learning paradigm revealed that the extinction of aversive memories was significantly enhanced in adult *Spig1*-KO mice (Figure 1B), while they showed a normal acquisition of aversive memories. Furthermore, spatial reference memory formation was also normal in the standard Morris water maze task.

An intracerebroventricular (icv) injection of anti-BDNF in the process of extinction learning transiently induced the recurrence of aversive memories in *Spig1*-KO mice, but demonstrated no effects in WT mice. These results indicate a critical role for SPIG1 in BDNF-mediated synaptic plasticity in the extinction of inhibitory avoidance memory.

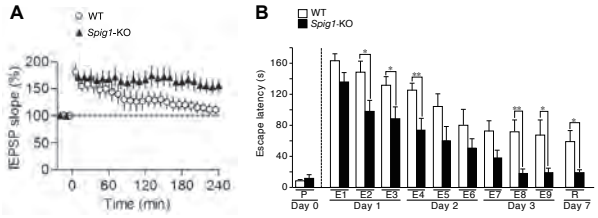

Figure 1. Enhanced LTP in the CA1 region of the hippocampus and enhanced extinction of aversive memories in Spig1-KO mice.
A) Plots of the field excitatory postsynaptic potential (fEPSP) slopes in WT and Spig1-KO mice. n = 9 from 6 mice.
B) Escape latency of WT and Spig1-KO mice. Spig1-KO mice exhibited facilitated extinction of inhibitory avoidance, irrespective of the normal acquisitive of aversive memories.
n = 6 or 7 for each group.

II. Physiological roles of receptor-like protein tyrosine phosphatases

Protein tyrosine phosphorylation plays numerous 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). However, the physiological functions and regulatory mechanisms of receptor-like PTPs (RPTPs) have not been 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

Eph receptors play a pivotal role in the axon guidance of retinal ganglion cells (RGCs) at the optic chiasm and the establishment of the topographic retinocollicular map. We previously demonstrated that protein tyrosine phosphatase receptor type O (PTPRO) is specifically involved in the control of retinotectal projections in chicks through the dephosphorylation of EphA and EphB receptors. We subsequently revealed that all the mouse R3 subfamily members (PTPRB, PTPRH, PTPRJ, and PTPRO) of the RPTP family inhibited Eph receptors as their substrates in cultured mammalian cells.

We investigated the functional roles of R3 RPTPs in the retinocollicular projection in mice. *Ptpro* and *Ptprj* were expressed in mouse RGCs; however, *Ptprj* expression levels were markedly higher than those of *Ptpro*. Consistent with their expression levels, Eph receptor activity was significantly enhanced in *Ptprj*-knockout (*Ptprj*-KO) retinas. In *Ptprj*-KO and *Ptprj*/*Ptpro*-double-KO (DKO) mice, the number of retinal axons that projected ipsilaterally or to the contralateral eye was significantly increased (Figure 2). Furthermore, retinal axons in *Ptprj*-KO and DKO mice formed anteriorly shifted ectopic terminal zones in the superior colliculus (SC).

We found that c-Abl (Abelson tyrosine kinase) was downstream of ephrin-Eph signaling for the repulsion of retinal axons at the optic chiasm (OC) and in the SC. c-Abl was identified as a novel substrate for PTPRJ and PTPRO, and the phosphorylation of c-Abl was upregulated in *Ptprj*-KO and DKO retinas. Thus, PTPRJ regulates retinocollicular projections in mice by controlling the activity of Eph and c-Abl kinases.

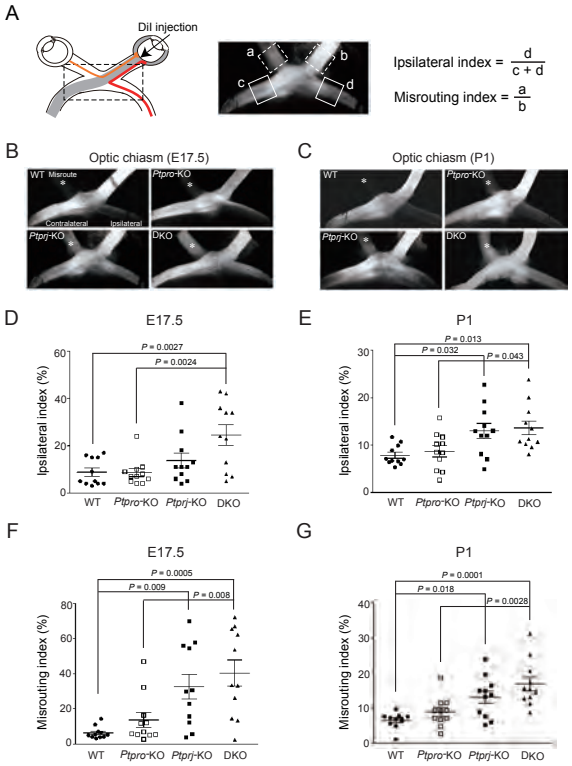

Figure 2. Ipsilateral projections of retinal axons at the OC in WT, *Ptpro*-KO, *Ptprj*-KO, and DKO mice.

A) Schematic representation of DiI tracing of retinal axons and quantification of the projection index to the ipsilateral side or contralateral eye. Retinal axons in the right eye were anterogradely labeled with DiI. Axons that projected ipsilaterally and misrouted to the contralateral eyes are indicated by red and orange lines, respectively. The ipsilateral index was calculated by dividing the fluorescent intensity of the ipsilateral optic tract by the total fluorescent intensity of both tracts. The misrouting index was calculated as the ratio between the fluorescent intensity of the left optic nerve and that of the right optic nerve.

B & C) Representative whole-mount ventral view of retinal axons at the OCs in WT, *Ptpro*-KO, *Ptprj*-KO, and DKO mice. Retinal axons in the right eye were labeled with DiI at E17.5 (B) and P1 (C). Arrows and asterisks indicate projections to the iplilateral side and contralateral optic nerve, respectively. Scale bars, 200 μm.

D & E) Index of projections to the ipsilateral side. n = 11 for each group.

F & G) Index of projections to the contralateral optic nerve. n = 11 for each group.

2-2. R5 RPTP subfamily

PTPRZ is one of the most abundant PTPs 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 the extracellular domain of PTPRZ is essential for achieving high-affinity binding sites for the endogenous ligands, such as pleiotrophin (PTN). We previously postulated that PTPRZ is a new molecular target in the development of drugs that treat glioblastoma and demyelinating diseases, such as multiple sclerosis (MS).

We have already revealed that PTPRZ functions to maintain OPCs in an undifferentiated state. We are now investigating (1) the overall picture of downstream signaling pathways involved in OPC differentiation, (2) the molecular basis of the PTN-induced inactivation of PTPRZ receptors, and (3) whether PTPRZ isoforms play a distinct physiological role by characterizing neurological phenotypes of two different knock-in mutant mouse strains carrying targeted loss of receptor functions of PTPRZ in comparison with *Ptprz*-null mice. Regarding (1), we recently reported that the PTN-PTPRZ signal activated the AFAP1L2-dependent PI3K-AKT pathway for oligodendrocyte differentiation (Tanga, N., *et al.*, Glia. doi: 10.1002/glia.23583).

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). These regions are known as brain loci that are substantially lacking a blood-brain barrier. We have already reported that Na_x signals in these brain regions are involved in the control of water and salt intake.

3-1. Central mechanisms of salt-induced hypertension through activation of sympathetic nerve activities

Hypertension is a major risk factor for cardiovascular disease worldwide, and approximately 40% (1 billion) of adults aged 25 and above have been diagnosed with hypertension (World Health Organization 2013). A positive correlation between salt (NaCl) intake and blood pressure (BP) has long been postulated. A battery of studies has shown that a diet high in salt increases sodium concentrations ($[Na^+]$) in plasma and the cerebrospinal fluid (CSF). $[Na^+]$ elevations in plasma and CSF enhance sympathetic nerve activity (SNA), leading to increases in BP. However, underlying mechanisms responsible for $[Na^+]$ sensing and signaling pathways to induce sympathetically-mediated BP elevations have not yet been elucidated.

We have recently revealed that sympathetic activation leading to BP increases was not induced in *Na_x*-KO mice by mandatory high salt (HS) intakes or the intraperitoneal/

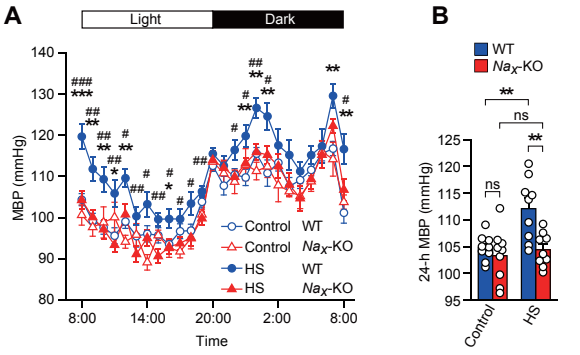

Figure 3. High salt (HS) ingestion induces an increase in $[Na^+]$ in body fluids that drives sympathetically mediated BP elevations in WT, but not in *Na_x*-KO mice.

A) Circadian changes in mean blood pressure (MBP) in control and HS-ingested mice. n = 9 mice for each. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (control WT versus HS-ingested WT); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (HS-ingested WT versus HS-ingested *Na_x*-KO).

B) Average MBP over a 24-hr period in control and HS-ingested mice. n = 9 for each.

intracerebroventricular infusions of hypertonic NaCl solutions, in contrast to WT mice (Figures 3 and 4). In the present study, we identified that Na_x channels in the OVLT play the role of a sensor detecting increases in [Na^+] in body fluids for BP control. In the OVLT, elevations in extracellular [Na^+] activated Na_x, and the Na^+ influx consequently leads to stimulation of anaerobic glycolysis in Na_x-positive glial cells to generate lactate. H^+ and lactate were then released from the glial cells through H^+/lactate symporters (monocarboxylate transporter, MCT). The released H^+ stimulated OVLT neurons projecting to the paraventricular hypothalamic nucleus (PVN) [OVLT(→PVN) neurons]. The H^+-dependent activation of OVLT(→PVN) neurons was mediated by acid-sensing ion channel 1a (ASIC1a) in the neurons. OVLT(→PVN) neurons activate PVN neurons and then rostral ventrolateral medulla (RVLM) neurons to increase SNA leading to BP elevations (Figure 5).

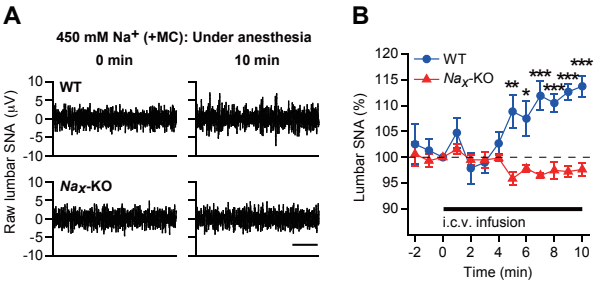

Figure 4. Lumbar SNA is stimulated by i.c.v. infusion of hypertonic Na solution in WT mice, but not in Na_x-KO mice.
A) A representative raw record of lumbar SNA just before (0 min) and 10 min after the i.c.v. infusion of hypertonic Na solution. Scale bar, 0.1 s. MC, Manning compound (a blocker of vasopressin receptor 1a).
B) Changes in lumbar SNA in response to the i.c.v. infusion of hypertonic Na solution. $n = 6$ each. The value at 0 min was set to 100%.

These molecular and cellular processes are the first steps in the activiation of the neurogenic mechanisms responsible for BP elevations in response to [Na^+] increases in the blood and CSF. Our results may provide novel neural therapeutic targets and encourage the future potential for treating a salt-sensitive phenotype in humans.

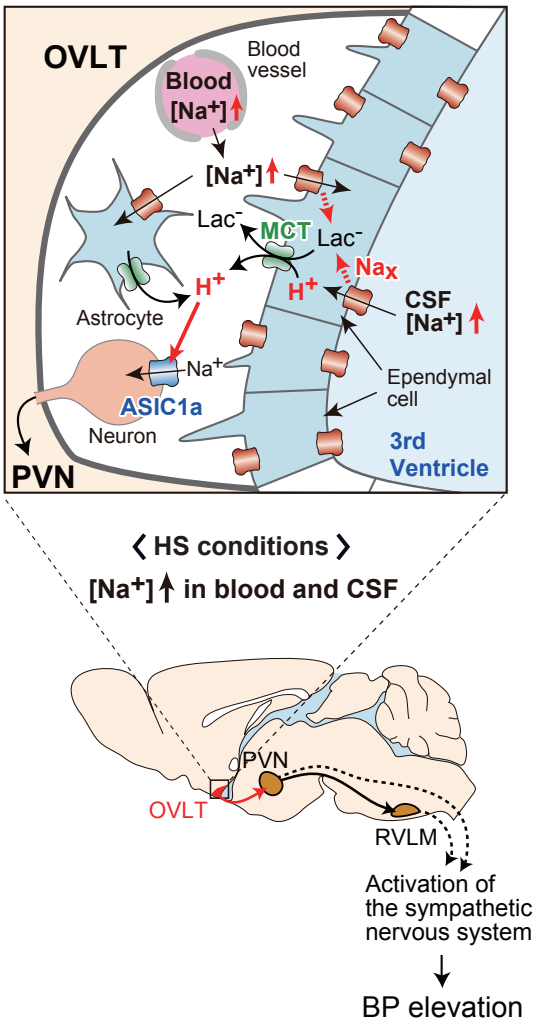

Figure 5. Central mechanisms of salt-induced BP elevations.
Upper: Increases in blood and CSF [Na^+] activate Na_x in the OVLT, and induce lactate (Lac^-) and H^+ release from Na_x-expressing ependymal cells through MCT. The resultant extracellular acidification (H^+) stimulates OVLT(→PVN) neurons via ASIC1a activation.
Lower: The OVLT-PVN-RVLM neural pathway is then activated and elevates BP through increases in SNA.

Publication List:

〔Original papers〕

- Suzuki, R., Fujikawa, A., Komatsu, Y., Kuboyama, K., Tanga, N., and Noda, M. (2018). Enhanced extinction of aversive memories in mice lacking SPARC-related protein containing immunoglobulin domains 1 (SPIG1/FSTL4). Neurobiol. Learn. Mem. *152*, 61-70.
- Yang, Y., Shintani, T., Takeuchi, Y., Shirasawa, T., and Noda, M. (2018). Protein tyrosine phosphatase receptor type J (PTPRJ) regulates retinal axonal projections by inhibiting Eph and Ab1 kinases in mice. J. Neurosci. *38*, 8345-8363.

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

- Nomura, K., Hiyama, T.Y., Sakuta, H., Matsuda, T., Lin, C.-H., Kobayashi, K., Kobayashi, K., Kuwaki, T., Takahashi, K., Matsui, S., and Noda, M. [Na^+] increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASIC1a. Neuron 2018 Nov 29.

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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 has been very difficult to reproducibly identify cell types. However, molecular genetic studies conducted over the past 15 years 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 in studying the 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 doing so 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 recording with relative ease using this transparent model. An additional advantage of zebrafish is that their CNS is much simpler than that of mammals. This enables us to perform detailed functional analysis of neuronal circuits at a single cell resolution. Our hope is to reveal the 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 experimental animals. Medaka have many advantages that are similar to those of zebrafish. Because NIBB is the main hub of the Medaka National Bioresource Project, we are ideally located in regards to 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 the CNS by using gene promoters/enhancers of genes and 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 genes whose expressions are tightly related to neurotransmitter properties of neurons (*i.e.*, vesicular glutamate transporter).

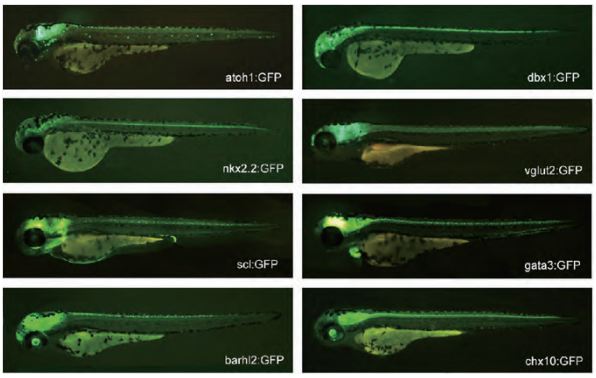

Figure 1. Examples of transgenic fish expressing GFP in specific classes of neurons.

In our early studies, we used a BAC-based transgenic technique for the generation of transgenic fish. By 2014, we succeeded in establishing a reliable knock-in method by utilizing the CRISPR-Cas9 system. The method we have developed is highly efficient, so much so that nearly one-third of the animals we raise become transgenic founders. Thus far, we have established more than 20 knock-in transgenic fish. Thus, this 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 the forelimbs and hindlimbs during locomotion. Neuronal circuits that control rhythmic limb movements in mammals have been investigated for decades, but our knowledge of them is still limited because of the complexity of their limbs. In this case, rhythmic movement of pectoral fins during swimming in larval zebrafish is an attractive model (Figure 2). The pectoral fins of larval zebrafish show left and right alternated rhythmic movements, and they are actuated only by two types of muscles: the abductor (Ab) and the adductor (Ad) (Figures 3). Due to the simplicity of pectoral fins, we expect that we will be able to characterize neuronal circuits that control rhythmic pectoral fins in more detail.

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

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

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

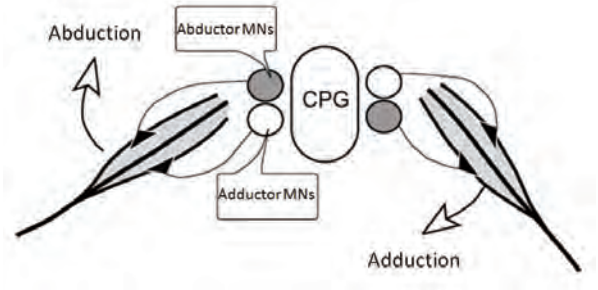

Figure 3. Schematic of rhythmic movements of pectoral fins during swimming. CPG, Central Pattern Generator.

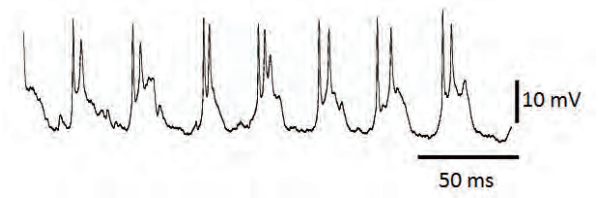

Figure 4. Rhythmic firings of an abductor motoneuron during swimming.

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

Inhibition plays an important role in shaping motor outputs during locomotion. In the spinal cord of larval zebrafish, there are mainly two types of inhibitory neurons: 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 an important role in ensuring antagonistic movements of the left and 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 had slowed down. The results show that En1-positive neurons play an important role for controlling locomotion speed.

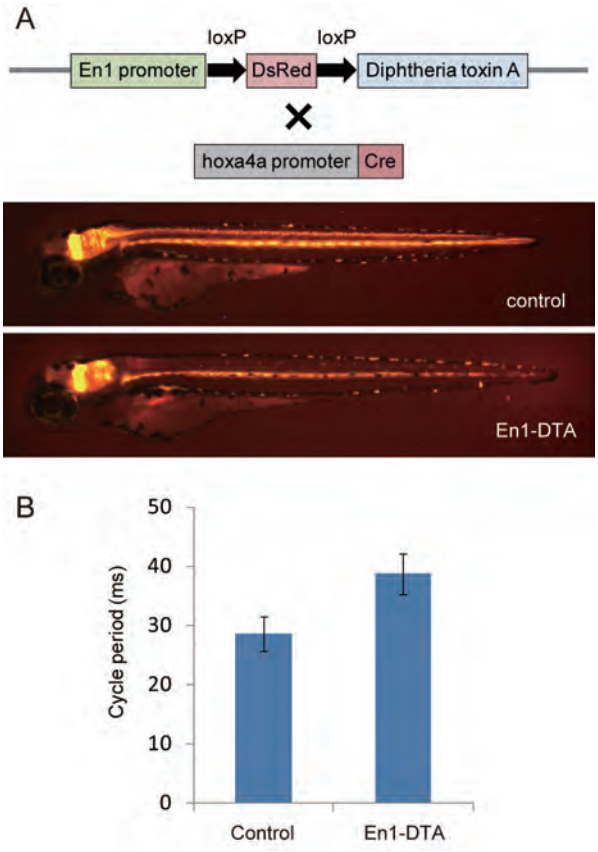

Figure 5. Spinal En1 neurons play an important role for controlling swimming speed. A, Ablation of spinal En1 neurons by using the Cre-loxP system. DTA, diphtheria-toxin A. B, Cycle period of the motor bursts during regular swimming in control and En1-DTA larvae.

IV. Behavioral role of the reciprocal inhibition between a pair of Mauthner cells during fast escapes in zebrafish

Vertebrates possess a bilateral CNS consisting of left and right sides of the brain and spinal cord. Whereas the bilateral CNS works symmetrically in some behaviors, in many cases it works asymmetrically between the left and right sides, such as when animals swim, walk, run, or perform lateralized movements. The reciprocal inhibition between the left and right sides is believed to play a key role in the asymmetrical activation of the bilateral CNS. However, despite the importance of reciprocal inhibition in the control of vertebrate behaviors, the identification of reciprocal inhibition circuits at the individual cell level and the contribution of each neuron to the asymmetric activity is still quite limited, largely because multiple circuits may participate in shaping motor output, making it difficult to identify circuit neurons, and to evaluate how each neuron contributes to producing these movements.

Teleost fish's fast escape response to sudden stimuli provides a typical lateralized behavioral model for investigating network organization and the function of reciprocal inhibition because the principal circuits from sensory inputs to motor outputs are simple and identifiable. The behavior is also basic enough that it is possible to evaluate the contribution of the key neurons. A pair of giant retic-

ulospinal neurons in the hindbrain, Mauthner (M) cells, are known to trigger the escape response, especially in response to sound/vibration stimuli. When one of the paired M-cell fires, it directly or indirectly activates the spinal motoneurons of the contralateral trunk muscles. Thus, the single spiking of an M-cell induces the contraction of trunk muscle exclusively along the axis on the contralateral side. However, sound/vibration stimuli may activate both M-cells because it is received by both ears directly as well as through the swim-bladder. Nevertheless, fish exhibit a C-bend and consistently escape in one direction. For that to occur, it has been believed that the reciprocal inhibition between the two M-cells play a critical role. It is known that there is a reciprocal inhibition between M-cells mediated by cranial relay neurons (CRNs) that receive excitatory inputs from M-axons and excite glycinergic interneurons contacting the contralateral M-cell. The role of the reciprocal inhibition for M-cell excitability and escape behavior, however, is unclear because the number and location of CRNs that mediate the reciprocal inhibition is unknown.

In the present study, we identified two paired CRNs in a GFP-expressing transgenic line of zebrafish larvae and examined the effects of their elimination on M-cell firing and sound/vibration-evoked escape behavior. We firstly demonstrated that two paired CRNs located in the posterior hindbrain largely mediate the reciprocal inhibition of M-cells, and secondly showed that the reciprocal inhibition plays a critical role in preventing bilateral firing of M-cells, thereby allowing for full flexion of the C-bend during escape.

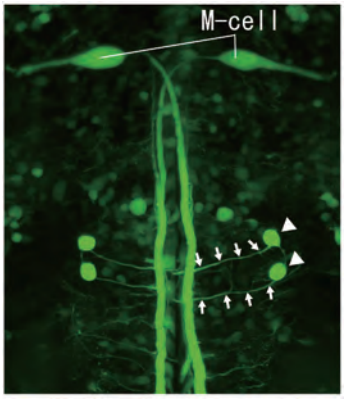

Figure 6. The Tol-056 enhancer trap line labels CRN neurons. In the enhancer trap line, GFP expression is present in a pair of M-cells in the hindbrain. In addition, GFP is expressed in a pair of two relatively large neurons (12-13 μm in diameter) in the caudal hindbrain (arrowheads). Prominent commissural axons arise from these two cells (arrows). These neurons are CRN neurons.

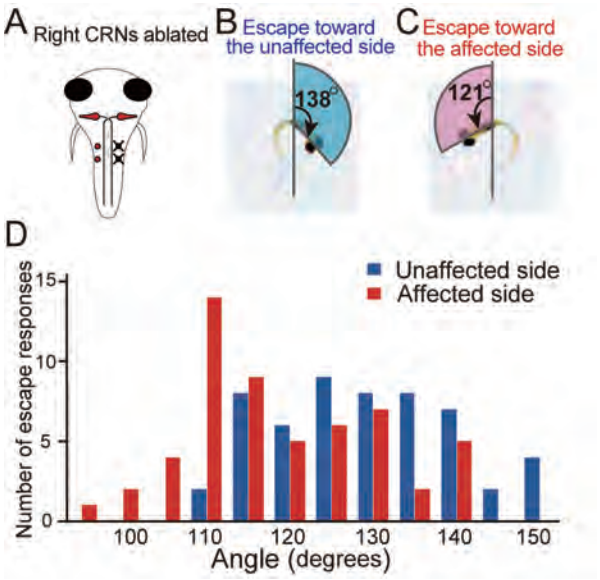

Figure 7. Behavioral experiments in the animals in which CRN neurons are unilaterally ablated. A, Schematic showing that CRN neurons on the right side are ablated. B, C, Examples of escape turns in an animal in which CRN neurons on the right side were ablated. (A) shows an example of a right turn (unaffected side), while (B) shows an example of a left turn (affected side). D, Histograms of the maximum bend angles collected from all the trials. Escapes turns toward the ablated side are categorized as “unaffected side” (blue) and those toward the opposite side are categorized as “affected side” (red).

Publication List:

〔Original Paper〕

- Watakabe, I., Hashimoto, H., Kimura, Y., Yokoi, S., Naruse, K., and Higashijima, S. (2018). Highly efficient generation of knock-in transgenic medaka by CRISPR/Cas9-mediated genome engineering. Zool. Lett. *4*, 3.

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

- Shimazaki, T., Tanimoto, M., Oda, Y., and Higashijima, S. Behavioral role of the reciprocal inhibition between a pair of Mauthner cells during fast escapes in zebrafish. J. Neurosci. 2018 Dec 23.

LABORATORY OF NEUROPHYSIOLOGY

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In order to interact successfully with their environments, animals must be able to comprehend their surroundings based on sensory information. Their visual system plays a particularly critical role in such environmental interactions.

The question of why we can see 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). We have recently made progress in studies of prey-predator interaction using these 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 played the role of the predator. Medaka exhibited predation behavior against several virtual characteristic plankton movements, particularly against a swimming pattern that could be characterized as a pink noise motion. Analyzing prey-predator interactions via pink noise motion will be a research field of great interest going into 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, and visual cues have been shown to play a crucial role in the formation of shoals. By 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 clearly 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 has been difficult to achieve in studies of their behavior. However, computer graphics allow us to systematically manipulate morphological and motion cues. Therefore, we have constructed 3D computer graphic animations based on tracking coordinate data and photo data obtained from real medaka. These virtual 3D models will allow us to represent medaka more 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). During this year, we have developed a system that presents interactive visual stimuli to fish, and a study using this system to examine the depth perception of fish is ongoing (Nishiumi *et al*., 2018).

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 the actual physical space. We developed a simple conceptual model 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 have successfully generated 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, Watanabe *et al*., 2018). These computer models of DNNs will help to facilitate our future work on perception science.

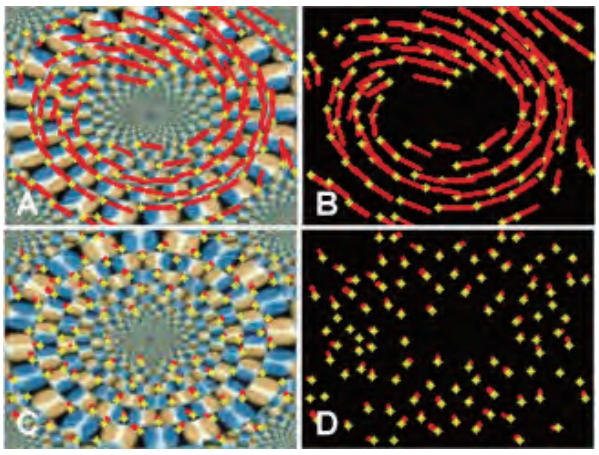

Figure 1. Optical flow vectors detected in the illusion. Red bars denote the direction and magnitude of vectors, yellow dots denote the start points of the vectors. A and B: Illusion, C and D: Non-Illusion.

Publication List:

〔Original papers〕

- Nishiumi, N., Matsuo, A., Kawabe, R., Payne, N., Huveneers, C., Watanabe, Y., Y., and Kawabata, Y. (2018). A miniaturized threshold-triggered acceleration data-logger for recording burst movements of aquatic animals. J. Exp. Biol. *221*, jeb172346.
- Watanabe, E., Kitaoka, A., Sakamoto, K., Yasugi, M. and Tanaka, K. (2018). Illusory motion reproduced by deep neural networks trained for prediction. Front. Psychol. *9*, 345.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

DIVISION OF EVOLUTIONARY BIOLOGY

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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, unexplained phenomena still remain, one of which is the evolution of complexity. It is difficult to explain the mechanisms needed to evolve complex adaptive traits at the 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 of them 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 identify the mechanisms needed for the evolution of complex characters.

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

The cell division axis has to be properly regulated during the development of both metazoans and land plants. Genetic changes in the regulation of the cell division axis lead to the development of multicellular organisms. Since they do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants most likely 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 identifying these factors, we also intend to study the spatiotemporal regulatory mechanisms will in order to understand the basis of body plan evolution in comparison to those found in the flowering plant *Arabidopsis thaliana* and the green algae *Closterium peracerosum–strigosum-littorale*.

Auxin is a phytohormone involved in many developmental processes in land plants. To reveal the relationship between auxin and cell division axis, Dr. Tsuyoshi Aoyama is leading the investigation of auxin dynamics during cell division and development. We analyzed promoter reporter and knock-in lines of auxin related genes involved in auxin synthesis, inactivation, transport, and distribution.

Dr. Ken Kosetsu and his colleagues found that one GRAS transcription factor is required for periclinal cell divisions, while another is required for anticlinal cell divisions. We identified that the former GRAS transcription factor represses the expression of the latter GRAS transcription factor. This regulation seems to decide the location where the division axis is changed from anticlinal to periclinal manner

From the observation of microtubule dynamics using the GFP-a-tubulin as a marker in the presence or absence of the GRAS transcription factor, Dr. Kosetsu's team found that the progression of the cell cycle was regulated by the GRAS transcription factor. This result suggests that the division axis is determined through the cell cycle-dependent cell shape.

Regulation of local cell growth underlies the geometric shape formation of individual cells. Cell shape is an instructive factor in oriented cell division, which guides morphogenesis in land plants. Mr. Liechi Zhang found a ABCB transporter mutant with cell shape abnormalities. This led to a retarded gametophore development of *Physcomitrella patens*. Time-lapse imaging of the fluorescent protein tagged transporter revealed a positive correlation between the membrane localization of this transporter and local cell growth. We are presently investigating the underlying mechanism using knock-out, knock-in, and inducible overexpression transgenic plants in wild type and cytoskeleton marker lines of *P. patens*.

This is a collaborative project that is being undertaken by our division and Dr. Rumiko Kofuji (Kanazawa University), Dr. Hiroyuki Sekimoto (Japan Women's University), and Atsushi Mochizuki (RIKEN).

III. Evolution of elaborated cell division machinery: Spindle body

During mitosis, all eukaryotic cells divide chromosomes into 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 land plants cells and

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their sister group, zygnematales green algae, form a bipolar spindle in the absence of centrosomes. In order to understand the mechanism of acentrosomal spindle formation, the steps of microtubule reorganization during spindle formation should be visualized. We collaborated with Prof. Tomomi Nemoto of 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. Analyses of microtubule behavior in the minispindle are underway in collaboration with Dr. Daisuke Tamaoki (Toyama Univ.) with Takashi Murata being the coordinating researcher for this study.

IV. Evolution of regeneration: Genetic 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. In the moss *Physcomitrella patens*, the leaf excision induces the reprogramming of differentiated leaf cells next to the excision to stem cells. We found that histone H3.3 chaperone HIRA proteins are induced in the leaf cells next to the excision, and positively regulate reprogramming. The role of HIRAs in reprogramming partly depends on the plant-specific transcription factor genes *SQUAMOSA PROMOTER BINDING PROTEINs* (*PpSBPs*). *PpSBPs* are involved in the repression of the reprogramming and HIRAs are necessary for the repression of *PpSBPs* through histone modifications during the reprogramming. Characterization of *HIRA*s and *PpSBPs* are in progress and are mainly being conducted by Yukiko Kabeya and Yosuke Tamada.

V. Evolution of regeneration: Master regulator for reprogramming *STEMIN*

Epigenetic modifications stabilize cell-specific gene expression programs to maintain cell identities in both metazoans and land plants. Notwithstanding the existence of these stable cell states, stem cells are formed from differentiated cells during post-embryonic development and regeneration in land plants. We have found a gene encoding a member of a plant-specific transcription factor, STEM CELL-INDUCING FACTOR 1 (STEMIN1) that was able to induce the direct reprogramming of differentiated leaf cells into chloronemal apical stem cells without wounding signals in *Physcomitrella patens*. To this end, we are investigating molecular mechanisms regulating changes in epigenetic modifications on STEMIN1-direct target genes after STEMIN1 induction. Masaki Ishikawa and Mio Morishita are this study's coordinating researchers.

VI. Evolution of regeneration: Other pathways

Nan Gu, a joint graduate student at both Huazhong Agricultural University and NIBB whose research interest is the relationship between DNA damage and the reprogramming from differentiated cells to stem cells, has found that DNA damage is a novel trigger to induce the reprogramming without wounding or dead cells in *P. patens*.

We found that INHIBITOR OF GROWTH (ING) proteins are involved in the stem cell formation of leaves with cuts in them. The ING proteins are known to regulate an apoptosis pathway in animals, but plants do not have this corresponding pathway.

VII. Evolution of molecular mechanisms of plant movement

The sensitive plant *Mimosa pudica* and the 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 these movements have been elucidated by physiological approaches, the lack of genetic tools available has hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we developed genetic transformation methods for these plants. Functional analysis of motor organ-enriched genes by CRISPR/Cas9-mediated knockout identified two channel genes and a transcription factor that play various roles in rapid leaf movements in *M. pudica*. We also generated a transgenic *D. muscipula* expressing a calcium sensor protein, which enables us to study how this plant counts the number of mechanical stimuli for its trap closure. The studies on *M. pudica* and *D. muscipula* were conducted mainly by Hiroaki Mano and Hiraku Suda, respectively.

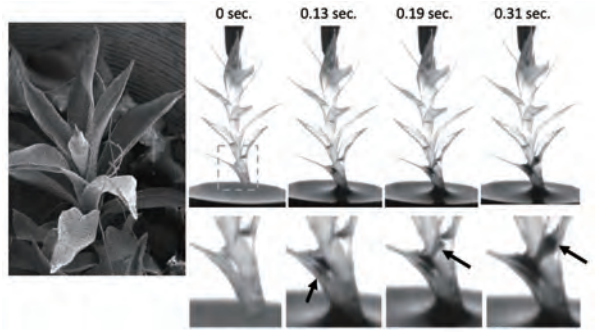

Figure 1. External water conduction of a gametophore. Water is transmitted from a leaf axil to the next leaf axil by capillarity.

VIII. Evolution of plant development 1

MIKC classic (MIKC^C)-type MADS-box genes encode transcription factors that function in various developmental processes, including angiosperm floral organ identity. Phylogenetic analyses of the MIKC^C type MADS-box family, including genes from non-flowering plants, suggest that the increased number of these genes in flowering plants is related to their functional divergence. However, their precise functions in non-flowering plants and their evolution throughout land plant diversification have been unknown to date. We found that MIKC^C-type MADS-box genes in the moss *Physcomitrella patens* function in two ways to enable fertilization. Analyses of protein localization, deletion mutants and overexpression lines of all six genes indicate that three MIKCC-type MADS-box genes redundantly regulate cell division and growth in the stems for appropriate external water conduction, as well as the formation of sperm

with motile flagella. The former function appears to be maintained in the flowering plant lineage, while the latter was lost in accordance with the loss of sperm.

IX. Evolution of plant development 2

Stem cells self-renew and produce precursor cells that differentiate to become specialised cell types. Land plants generate several types of stem cells that give rise to most organs of the plant body and whose characters determine the body's organisation. The moss *Physcomitrella patens* forms eight types of stem cells throughout its life cycle. Under gametangium-inducing conditions, multiple antheridium apical stem cells are formed at the tip of the gametophore and each antheridium apical stem cell divides to form an antheridium. We found that the gametophore apical stem cell, which typically forms leaf and stem tissues, changes to become a new type of stem cell, which we term the antheridium initial stem cell. This antheridium initial stem cell produces multiple antheridium apical stem cells, resulting in a cluster of antheridia at the tip of gametophore. This is the first time that a land plant stem cell directly producing another type of stem cell during normal development has been reported. Notably, the antheridium apical stem cells are distally produced from the antheridium initial stem cell, similar to the root cap stem cells of vascular plants, suggesting the use of similar molecular mechanisms and a possible evolutionary relationship. This research is a joint collaboration with Dr. Rumiko Kofuji in Kanazawa University.

Changxiu Yu, a joint graduate student of both Huazhong Agricultural University and NIBB whose research interest is the conversion of the gametophore stem cell to the antheridium initial stem cell induced by environmental changes, is trying to identify genes involved in the abovementioned process.

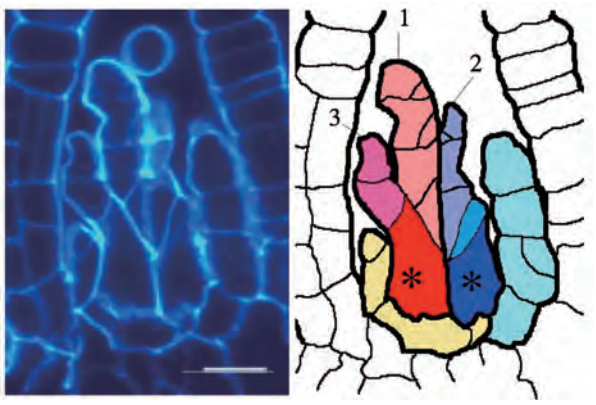

Figure 2. Cell divisions of antheridium initial stem cells (*).Numbers from 1 to 7 indicate the first to the seventh antheridium apical stem cells.

X. Evolution of plant development 3

To investigate the evolution of novel complex traits, the following studies are being undertaken by graduate students: Chiharu Kamida is studying genes involved in movable tentacle development in the sundew *Drosera spatulata*. The pseudanthium is a flower-like inflorescence, whose molecular development mechanisms 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 is investigating the evolution of signaling pathways of the phytohormone strigolactone in land plants.

XI. Evolution of carnivory in flowering plants

Carnivorous plants exploit animals as a source of nutrition and have inspired long-standing questions about the origin and evolution of carnivory-related traits. To investigate the molecular bases of carnivory, Dr. Hideki Narukawa performed mostly comparative analysis of carnivorous pitcher leaves and non-carnivorous flat leaves in the carnivorous plant *Cephalotus follicularis*. We found that hollow formation, which was the first step in pitcher leaf development, was initiated by growth inhibition on the adaxial side of leaf primordia. This process may be regulated by the phytohormone cytokinin.

In order to elucidate the origin of the pitcher shape, Gergo Palfalvi, a graduate student, looked into the initiation factors separating the flat leaf and pitcher leaf establishment in the primordia. Mass sequencing of shoot apices among several environmental conditions utilized to alter the leaf/pitcher ratio are in progress. We are also working on refinement of the genome especially in epigenetic studies.

Publication List:

〔Original papers〕

- Kofuji, R., Yagita, Y., Murata, T., and Hasebe, M. (2018). Antheridial development in the moss *Physcomitrella patens*: implications for understanding stem cells in mosses. Philos. Trans. R. Roc. Lond. B Biol. Sci. *373*, 20160494.
- Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T. and Hasebe, M. (2018). Physcomitrella MADS-box genes regulate water supply and sperm movement for fertilization. Nat. Plants *4*, 36-45.

〔Review paper〕

- Renner, T., Lan, T., Farr, K.M., Ibarra-Laclette, E., Herrera-Estrella, L., Schuster, S.C., Hasebe, M., Fukushima, K., and Albert, V.A. (2018). Carnivorous plant genomes. In: Carnivorous Plants: Physiology, Ecology, and Evolution, A.M. Ellison and L. Adamec, eds. (Oxford University Press), pp. 135-153.

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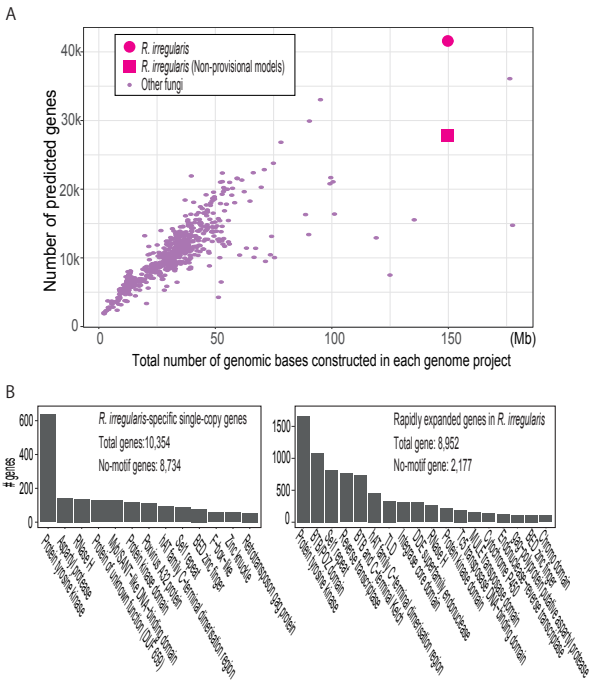

Figure 1. Gene expansion in *R. irregularis*. (A) Total assembly size and predicted gene number in fungal genomes. The assembly statistics (768 fungal species) were obtained from "ftp://ftp.ncbi.nlm.nih.gov/genomes". (B) The number of expanded *R. irregularis* genes having any Pfam motifs. Minor motifs (<50 or <100) were omitted from the figure.

Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on earth. In this symbiosis, soil bacteria called rhizobia supplies 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. However, 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 Improved genome sequence of an arbuscular mycorrhizal fungus, *Rhizophagus irregularis*

Arbuscular mycorrhizal fungus (AMF) species are some of the most widespread symbionts found in land plants. Our much improved reference genome assembly of a model AMF, *Rhizophagus irregularis* DAOM-181602 (NSDC# = BDIQ01000000), facilitated the discovery of ribosomal RNA genes (rDNA) with unusual characteristics, *i.e.* absence of tandem repeat structure and intragenomic homogeneity. RNA-seq analysis confirmed that all rDNA variants are actively transcribed. Observed polymorphism may modulate translation by using different ribosomes, which in itself depends on biotic and abiotic interactions.

Our gene model construct then showed *R. irregularis* has one of the largest numbers of genes in fungi (41,572 gene models, Figure 1A). Ortholog analyses indicate that this gene number increase was caused by lineage-specific expansions of gene families and not by whole-genome duplications. The motif annotation indicates that inflated genes may contribute to the signaling pathways of AMF species. The most frequently observed domain motif in the expanded gene families was protein tyrosine kinase PF07714, which is often found in signaling proteins in multicellular organisms. Other signal-related motifs (*e.g.* Sel1 repeat and BED zinc finger) were also found in the inflated genes (Figure 1B). This inflation of signaling-related genes may have led the development of a complex signaling pathway in AMF (*e.g.*, establishment of symbiosis with pathways via SIS141 and lyso-phosphatidylcholine). Additionally, we detected highly duplicated ribosomal protein genes (*e.g.* 25 paralogs for ribosomal protein S17/S11) which may also account for the heterogeneity of ribosomes.

1-2 Genome sequencing of a non-model arbuscular mycorrhizal fungus *Rhizophagus clarus* and comparative analysis with other fungi

Mycorrhizal symbiosis is one of the most fundamental types of mutualistic plant-microbe interaction, and the arbuscular mycorrhizae have the most general symbiotic type and boasts the longest history. However, AMF genomes were not well characterized because genome sequencing was available for only one model species, *R. irregularis*. We sequenced the genome of the AM fungus *R. clarus* HR1, and compared the sequence with the genome sequence of other fungi, including the model AMF *R. irregularis*.

We constructed a total 116.41 Mbp of draft genome sequence (Genbank: BEXD01000001 - BEXD01004424). Comparative analysis revealed pathways that were commonly missing from the genomes of two AMF species

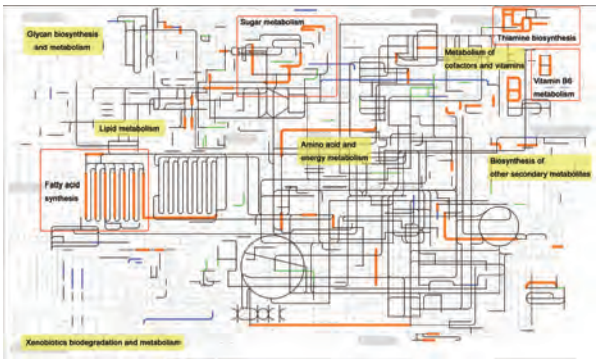

Figure 2. Missing metabolic pathways of two *Rhizophagus* species. The thick orange lines indicate enzyme pathways whose genes are absent in two AMF but present in *Saccharomyces* and *Aspergillus*.

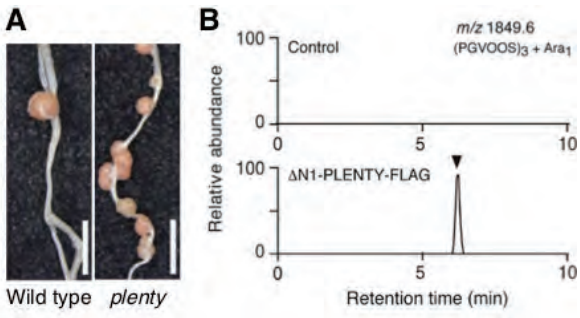

Figure 3. (A) Nodulation phenotype of wild-type and *plenty*. 14 days after inoculation with rhizobia. Bars=2 mm. (B) Identification of the HPAT activity of PLENTY *in vitro*. The synthetic substrate peptide (PGVOOS)3 was incubated with the recombinant PLENTY protein in the presence of UDP-β-l-Araf. Arrowheads indicates a peak of the arabinosylated substrate detected by LC/MS.

(Figure 2). Between the two of them, an absence of cytosolic fatty acid synthase (FAS) is an important feature because long-chain fatty acids are fundamental in membrane construction. In AMF genomes, only the mitochondrial FAS pathway, which synthesizes respiratory cofactors, was found. The absence of genes encoding enzymes for several other metabolic pathways was also identified in the two AM fungi, including thiamine biosynthesis and the conversion of vitamin B6 derivatives.

We also found that a large proportion of the genes encoding glucose-producing polysaccharide hydrolases appear to be absent in AM fungi. Most of these pathways are maintained in other biotrophic fungi, including ectomycorrhizal and pathogenic fungi. An absence of these enzymes shows that AMF has an extremely low capacity in regards to utilizing environmental polysaccharides.

Missing genes for enzymes in primary metabolic pathways imply that AM fungi may have a higher dependency on host plants than other biotrophic fungi. These missing metabolic pathways are supposed to be the result of a long-lasting symbiotic lifestyle. This information also provides a genetic basis to explore the physiological characteristics and auxotrophy of AM fungi.

II. Nodule symbiosis

PLENTY, a hydroxyproline O-arabinosyltransferase, negatively regulates root nodule symbiosis

The nodule symbiosis is beneficial for host legumes. However, forming nodules and nitrogen-fixation consume energy. Therefore, legumes strictly control nodule numbers to keep the balance between cost and benefit through a shoot-mediated long-distance negative feedback system, known as autoregulation of nodulation (AON).

Our previous studies showed that small peptides CLAVATA3/ESR (CLE)-RELATED-ROOT SIGNAL1 (CLE-RS1), CLE-RS2, and CLE-RS3 serve as root-derived mobile signals in AON. They are synthesized in roots in response to rhizobial infection and translocated to the shoot via xylem vessels. CLE-RS2 detected in xylem sap was arabinosylated within a hydroxyproline residue conserved among these small peptides. An ectodomain of a shoot-acting receptor protein kinase, HYPERNODULATION AND ABERRANT ROOT FORMATION1 (HAR1), efficiently binds with arabinosylated CLE-RS2. This post-translational modification is essential for its function of suppressing nodulation in the HAR1-dependent manner.

L. japonicus plenty mutants exhibit an excessive production of root nodules and short roots. (Figure 3A). This hypernodulation phenotype resembles that of *har1* mutants. Map-based cloning revealed that the responsible gene encodes a hydroxyproline O-arabinosyltransferase widely conserved in plants. PLENTY was predicted to have a secretory signal and a transmembrane domain at the amino-terminal region. Consistent with this prediction, PLENTY that was expressed in tobacco leaves localized to the Golgi network. Furthermore, microsomal fractions of yeast cells expressing PLENTY protein efficiently arabinosylated a synthetic substrate *in vitro* (Figure 3B). Thus, PLENTY possesses a hydroxyproline O-arabinosyltransferase activity. Overexpression of CLE-RS peptide precursors strongly inhibited nodulation in wild-type plants, whereas this inhibitory effect was attenuated in *plenty* mutants, suggesting involvement of PLENTY in AON downstream of CLE-RS expression. If one were to consider the lower effectiveness of CLE-RS peptides in *plenty*, they are strong candidates as PLENTY substrates. We discovered a novel factor involved in post-translational modification in AON.

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

Self-organization of spatially regular patterns is critical for the development and differentiation of multicellular organisms. Phyllotaxis, the arrangement of leaves on a plant stem, shows diverse patterns that differ according to plant species and is an area of great interest for many researchers because of its beautiful geometric configuration (Figure 4A). In particular, it is well known that the spiral phyllotaxis is closely related to mathematical concepts such as the golden ratio and Fibonacci sequence. While phyllotaxis pattern is established by the mutual interaction between a diffusible plant hormone auxin and its efflux carrier PIN1 (Figure 4A), its molecular mechanism 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 (Figure 4B, Model O). 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 (Figure 4B, Model A). To unravel this mechanism, we identified 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 (Figure 4B, Model B). 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.

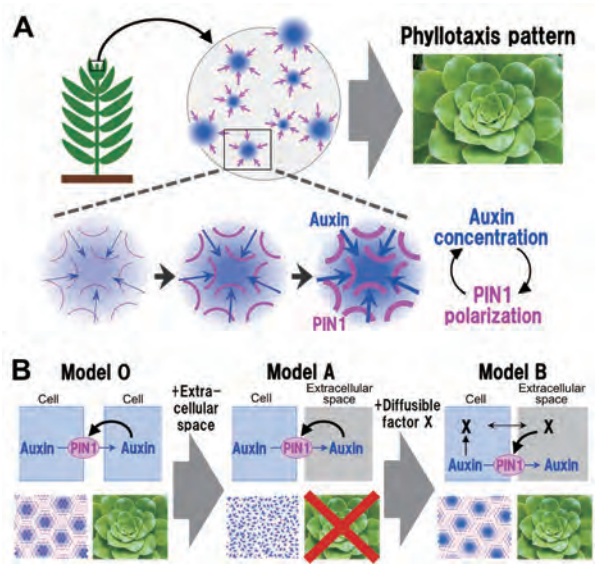

Figure 4. (A) Phyllotaxis is well known because of its beautiful geometric configuration, which is derived from the constant spacing between leaf primordia. This phyllotaxis is established by mutual interaction between a diffusible plant hormone auxin and its efflux carrier PIN1, which cooperatively generate a regular pattern of auxin maxima, small regions with high auxin concentrations, leading to leaf primordia. (B) Spatial regularity of auxin maxima in Model O is completely disrupted by introducing the extracellular space (Model A), but can be restored by assuming a diffusible molecule that mutually interacts with auxin–PIN1 dynamics (Model B).

Publication List:

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- Fujita, H., and Kawaguchi, M. (2018). Spatial regularity control of phyllotaxis pattern generated by the mutual interaction between auxin and PIN1. PLoS Comput. Biol. *14*, e1006065.
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- Nishida, H., Tanaka, S., Handa, Y., Ito, M., Sakamoto, Y., Matsunaga, S., Betsuyaku, S., Miura, K., Soyano, T., Kawaguchi, M., and Suzaki, T. (2018). A NIN-LIKE PROTEIN mediates nitrate-induced control of root nodule symbiosis in *Lotus japonicus*. Nat. Commun. *9*, 499.
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〔Original paper (E-publication ahead of print)〕

- Yoro, E., Nishida, H., Ogawa-Ohnishi, M., Yoshida, C., Suzaki, T., Matsubayashi, Y., and Kawaguchi, M. PLENTY, a hydroxyproline O-arabinosyltransferase, negatively regulates root nodule symbiosis in *Lotus japonicus*. J. Exp. Bot. 2018 Oct 23. doi: 10.1093/jxb/ery364.

〔Review articles〕

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DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY

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	KIYOHARA, Megumi

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 that lead to the large variety of traits that they display. From this wealth of exciting traits, our lab is currently focused 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, the flight organ of insects has uniquely evolved. Despite over two centuries of debate, the evolutionary origin of the insect wing is still an enigma. We are trying to uncover it by 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, which is phylogenetically the closest extant relative of winged (pterygote) insects, thus making it ideal for elucidating wing origin. We cloned *vg* and *sd* orthologs from *T. domestica* (*Td-vg* and *Td-sd*),and we developed RNA interference (RNAi) based methods for *T. domestica* to examine the functions of these genes. We are currently testing for the 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 (Figure 1B) 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 the mealworm beetle (Ohde *et al*., 2013). Based on these facts, we hypothesize that ancestral lateral body wall outgrowths evolved into functional wings. However, genetic tools available for the analysis of basally branching wingless species are limited. To overcome these limitations, we established CRISPR/Cas9-based germline genome editing in *T. domestica*. Heritable mutations were successfully introduced in *white* locus, an evolutionarily conserved gene, encoding the ATP-binding cassette (ABC) membrane transporter, of *T. domestica* by using CRISPR/Cas9 system, which results in white-eyed firebrats. In addition to the RNAi-mediated gene knockdown (Ohde *et al*., 2009), germline genome editing using CRISPR/Cas9 in *T. domestica* provides a platform technology that opens new research opportunities on the evolution of insects, such as on the insect wing origin. We are now conducting gene knock-out/in within various "wing" genes to identify details genetic and cell lineage analyses in *T. domestica* (Figure 1).

II. Wing color patterns and mimicry of ladybird beetles

A tremendous range of diversity of wing color patterns have evolved within insects, which play various ecologically important roles such as intraspecific sexual signaling, mimesis, mimicry, and warning against predators. However, the molecular mechanisms responsible for generating such color patterns in most ladybird species 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

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

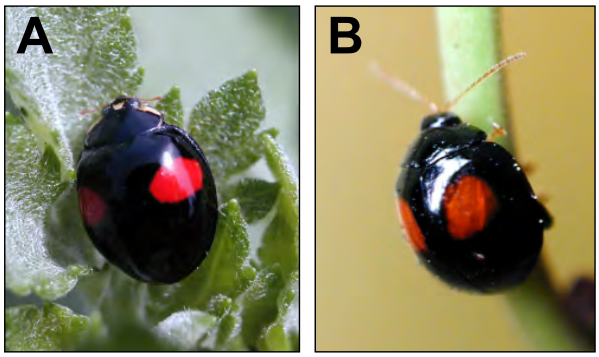

Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

and variable wing color patterns consisting of black and red pigments (Figure 2A). The ladybird's vivid wing color pattern functions as a warning signal to predators that they taste bad. At the same time, various other insect species utilize 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 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 transformation using a *piggyBac* vector and RNAi in the ladybirds.

We recently identified a key gene, *pannier*, which regulates intraspecific color pattern polymorphism in *H. axyridis* using next generation sequencing technologies (RNA-seq and *de novo* genome assembly), and an RNAi-based screening method that we established. *pannier* is expressed in specific regions in the wing, which will synthesize black pigment, and suppress red pigmentation. The expression pattern of *pannier* is diversified according to the diverse color pattern types in *H. axyridis*. These findings suggest that regulatory shift, such as change in enhancer activity, at the *pannier* locus may be crucial for the evolution of wing color patterns in *H. axyridis*. We are currently trying to elucidate the evolutionary origin of color patterns in ladybirds focusing on regulatory shifts at the *pannier* loci. We are also establishing genome-editing technologies using TALEN and CRISPR/Cas9 to tackle this issue, 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. We are also establishing cryopreservation methods for germline cells in ladybird beetles to overcome laborious tasks and the high risk of losing valuable genetic bioresources in non-model insects. We recently established ovary transplantation and ovarian cryopreservation techniques in ladybird beetles. We hope that the genetic tools and techniques that we have established will facilitate the ladybird beetle's research.

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 a subject of great potential 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 are focusing on the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns. The male-specific horns of *T. dichotomus* are one of the best models to study how an extreme, sex-specific morphology is formed (Figure 3, Control). We have developed a technique for larval RNAi in *T. dichotomus*, allowing 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 the 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*, the developing horn discs' mRNA has been assessed by deep-sequencing transcriptome analysis (RNA-seq). We narrowed down the genes associated with horn formation to 49 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 (*SP8, pannier, Rx, Optix, BarH1, Tbx20, Sox21b, dachshund, Scr, Sox14, abrupt*) 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 (Figure 3).

To understand how beetle horns have molecularly 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.

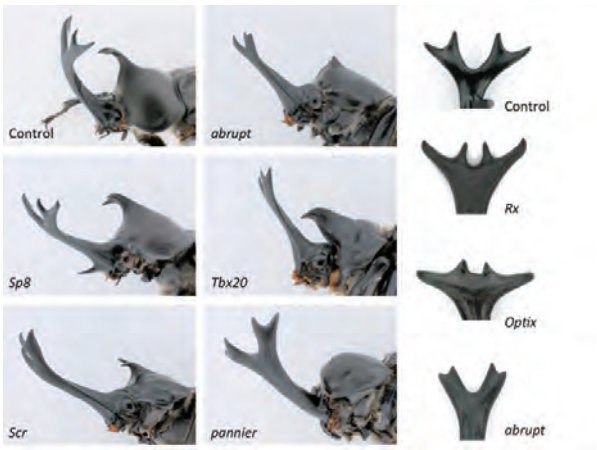

Figure 3. RNAi-mediated gene knockdown alters horn shapes and sizes in head and thorax of the Japanese rhinoceros beetle, *Trypoxylus dichotomus*.

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- Tsuji, T., Gotoh, H., Morita, S. Hirata, J., Minakuchi, Y., Yaginuma, T., Toyoda, A., and Niimi, T. (2018). Molecular characterization of eye pigmentation-related ABC transporter genes in the ladybird beetle *Harmonia axyridis* reveals striking gene duplication of the *white* gene. Zool. Sci. *35*, 260-267.
- Zinna, R.A., Emlen, D.J., Lavine, L.C., Johns, A., Gotoh, H., Niimi, T., and Dworkin, I. (2018). Sexual dimorphism and heightened conditional expression in a sexually selected weapon in the Asian rhinoceros beetle. Mol. Ecol. *27*, 5049-5072.

〔Review article〕

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LABORATORY OF BIORESOURCES

Specially Appointed Professor
NARUSE, Kiyoshi

Assistant Professor:	*ANSAI, Satoshi*
Postdoctoral Fellow:	*KAJIURA-KOBAYASHI, Hiroko*
Research Staff:	*KANEKO, Hiroyo*
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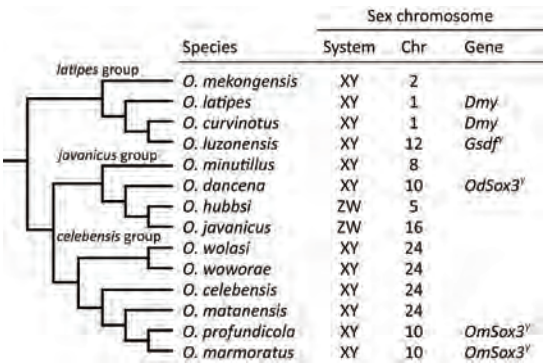
	TAKAKI, Chikako
	TESHIMA, Yuuko
	TORII, Naoko
	YAMAZAKI, Toko
	YANOGAWA, Azusa
Secretary:	*SUZUKI, Tokiko*

The 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 their 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 was charged with the responsibility of leading 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 the sexual development of organisms. 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, thus suggesting that sex determination mechanisms are regulated by different genes and have evolved rapidly.



Species	Sex chromosome System	Chr	Gene
O. mekongensis	XY	2	
O. latipes	XY	1	*Dmy*
O. curvinotus	XY	1	*Dmy*
O. luzonensis	XY	12	*Gsdf*^{Y}
O. minutillus	XY	8	
O. dancena	XY	10	*OdSox3*^{Y}
O. hubbsi	ZW	5	
O. javanicus	ZW	16	
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.

Medaka fish in the genus *Oryzias* have different sex chromosomes with different systems (XY and ZW), therefore 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. We recently identified *Sox3* as a novel sex-determining gene on the XY sex chromosomes in the marine medaka *Oryzias dancena/melastgma* through positional cloning. Sex reversed phenotypes in transgenic fish and loss-of-function mutants in the Y chromosomal *Sox3* allele both point to its critical role in sex determination, suggesting that the neo-Y chromosome of *O. dancena* arose by the co-option of *Sox3*. Furthermore, we also found the *Sox3* gene 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 the *Oryzias* species is polyphyletic, and the Y-specific insertion has not been found in *O. dancena*, indicating that *Sox3* has evolved as the sex-determining gene independently in different lineages of *Oryzias*. These results suggest that *Sox3* might have continuously and independently acquired the novel male-determining function during vertebrate evolution.

II. Identification of the causal gene of the medaka body color mutant, *few melanophore (fm)*

The body coloration of animals is due to pigment cells derived from neural crest cells, which are multipotent and differentiate into diverse cell types. Medaka (*O. latipes*) possess four distinct types of pigment cells known as melanophores, xanthophores, iridophores, and leucophores. The *few melanophore* (*fm*) mutant found amongst medaka is characterized by reduced numbers of melanophores and leucophores. We have identified kit-ligand as the gene whose mutation gives rise to the *fm* phenotype. This identification was confirmed by generation of kit-ligand knockout medaka and the findings that these fish also manifest reduced numbers of melanophores and leucophores and fail to rescue

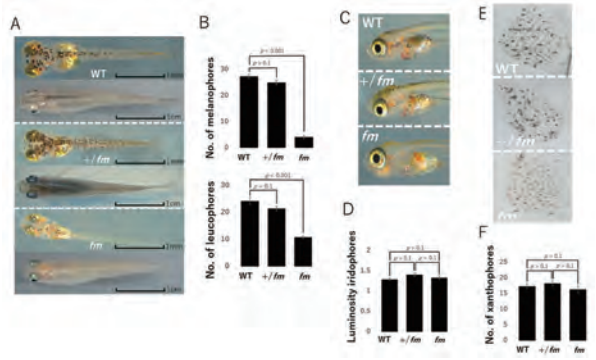


Figure 2. The *fm* mutants show a reduction in melanophore and leucophore pigmentation (A and B) and not in xanthophore and iridophore pigmentation (C, D, E and F).

the *fm* mutant phenotype. We also found that expression of *sox5*, *pax7a*, *pax3a*, and *mitfa* genes is down-regulated in both *fm* and *kit-ligand* knockout medaka, implicating c-Kit signaling in the regulation of the expression of these genes as well as the encoded transcription factors in pigment cell specification.

Our results may provide insight into the pathogenesis of c-Kit–related pigmentation disorders such as piebaldism in humans, and our kit-ligand knockout medaka may prove useful as a tool for drug screening.

III. Genome sequence of the Javanese medaka, *Oryzias javanicus*, as a model for studying seawater adaptation

Medaka fish in the genus *Oryzias* are an emerging model system for studying the molecular basis of vertebrate evolution. This genus contains approximately 35 species and exhibits great morphological, ecological and physiological differences among its species. Among these species, the Javanese medaka, *Oryzias javanicus*, is the species that has most typically adapted to seawater. We sequenced and assembled the whole genome of *O. javanicus*, as a model fish species for studying molecular mechanisms of seawater adaptation. In teleost fish, the major osmoregulatory organs are the gills, intestines and kidneys, and these play different roles to maintain body fluid homeostasis. Many genes encoding hormones, receptors, osmolytes, transporters, channels and cellular junction proteins are potentially involved in this osmotic regulation. In addition to the osmoregulation, hatching enzyme activity dramatically changes in different salt conditions. At the hatching stage, fish embryos secrete a specific cocktail of enzymes in order to dissolve the envelope. In the medaka *O. latipes*, digestion of the envelope occurs after the cooperative action of two kinds of hatching enzymes: (i) the high choriolytic enzyme (HCE) and (ii) the low choriolytic enzyme (LCE) (Yasumasu *et al*., 2010). The HCE shows higher activity in freshwater than in brackish water (Kawaguchi *et al*., 2013). Thus, availability of the high-quality reference genome in *O. javanicus* would facilitate further research for investigating the molecular basis of physiological differences, including the osmotic regulation and the hatching enzyme activity among *Oryzias* species.

IV. Adrianichthyidae fish in Sulawesi: a model system to explore the molecular genetic basis of diversification in sexual dimorphism

Sexual dimorphism is prevalent, but often differs remarkably between closely related species. However, we know little about which genes and genetic changes can actually contribute to diversification of sexually dimorphic traits. The family Adrianichthyidae are a small teleost species commonly considered to be medaka. Although their native habitats are widely distributed in East and South-East Asia, 20 of 37 Adrianichthyidae species are endemic to Sulawesi, Indonesia. We have studied the molecular genetic basis of diversification in sexual dimorphism using the endemic species as a model system, because their sexual dimorphic body colorations are significantly diversified in closely-related species. As a research resource for the Sulawesi species, we firstly sequenced and assembled a genome of *Oryzias celebensis*. The assembly was anchored to 18 different chromosomes by linkage mapping and was annotated using RNA-seq data from adult and embryonic tissues. We then investigated the molecular mechanisms underlying red coloration in pectoral fins, a characteristic feature of *O. woworae* males. Quantitative trait loci (QTL) mapping in a F2 intercross between a male of *O. woworae* and a female of a close relative *O. celebensis* without any red fins revealed that an autosomal locus controls the red pigmentation. Subsequent quantitative gene expression analysis revealed that a gene is a strong candidate responsible for the red fins, which is highly expressed in the red fins of *O. woworae* males by the cis-regulatory mutation. CRISPR/Cas-mediated mutagenesis in the gene caused a deficiency in the fins' pigment cells. Additionally, a behavioral analysis showed that the mutant males had lower reproductive success than the wild-type males. These results suggest that ectopic expression of an autosomal gene in the pectoral fins by the cis-regulatory change will cause the red coloration in the pectoral fins, which might spread by increasing the reproductive success in males.

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

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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 subject 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 during the course of morphogenesis.

The outline shape of an adult wing is often different from that of a 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 has been shown that the dying cells in the degeneration region have characteristics in common with 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. At that time, the macrophages are excluded from the differentiation region because the basal surfaces of the dorsal and ventral epithelium strongly adhere to each other in the differentiation region. The concentration of macrophages to the degeneration region seems to accelerate the removal of the dead cells and the shrinkage of the degeneration region.

A possible physiological role of cell degeneration at the wing margin is to make space for the growth of the marginal scales. Marginal scales are remarkably 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 the bristles growing among them (Yoshida and Emoto, Zool. Sci. *28*, 430-437, 2011).

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

In small moths which have very long scales along their wing margins, the cuticle of the pupal wing does not appear to be large enough to house these scales. We examined the developmental processes of the pupal wings of three species of small gelechiid moths and found that a shrinkage of the differentiation region occurs which has not been observed in large winged butterflies and moths. This phenomenon is concomitant with the programmed cell deaths at the wing margin, and 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* have 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).

III. Transparent wing formation by scale removal

The wings of butterflies and moths are usually fully covered with scales but in some species the adult wing lacks scales in some parts, thus giving the wing a transparent appearance.

We studied the emergence process of the *Cephonodes hylas* moth and found that 1) the future transparent part of the pupal wing is covered with scales that are morphologically different from the scales found on the other part and 2) the young adult moth just after emergence strongly vibrates its wing which loosens the scales of the future transparent part until the moth flies away. This results in the transparent wing leaving scales behind. The large size and the shape containing a small peg, which stabs the socket on the wing plane holding the scale in place, found on the scales of the future transparent part are key feature in enabling the formation of the transparent wing (Yoshida *et al*., in preparation).

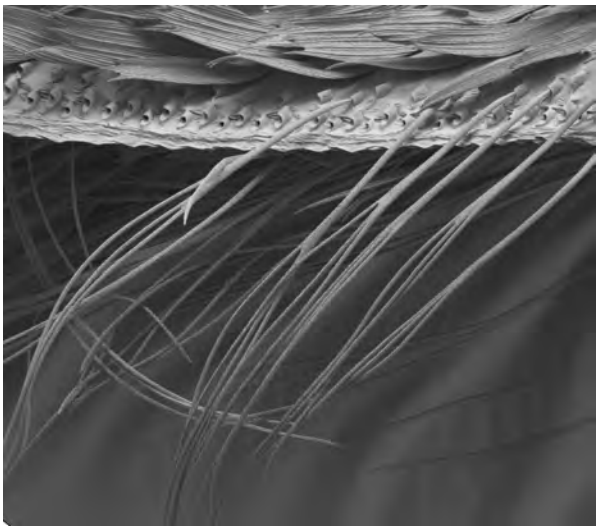

Figure 1. Scanning electron microscopy of the wing margin of *P. operculella*. Long marginal scales show branching morphology.

LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Amino acids are the most important nutrients used in protein building; therefore, their perception is essential for all cells' existence. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. On one hand, TORC1 is involved in amino acid sensing, regulation of 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 TOR signaling pathway branches (Figure 1). Recently, we found that tRNA plays a pivotal role in the ability of TORC1 to sense amino acids (Figure 1).

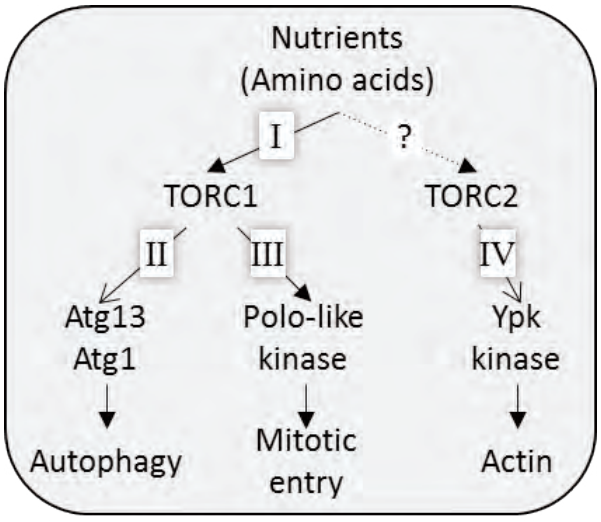

Figure 1. Tor signaling pathway in budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

TORC1 is regulated by amino acids which in themsleves are fundamental nutrients. 20 species of amino acids building proteins cannot be interchanged with each other. Therefore, each amino acid must be individually detected by TORC1.

We conducted genetic research and discovered 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 was proposed that contends 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 (Figure 2).

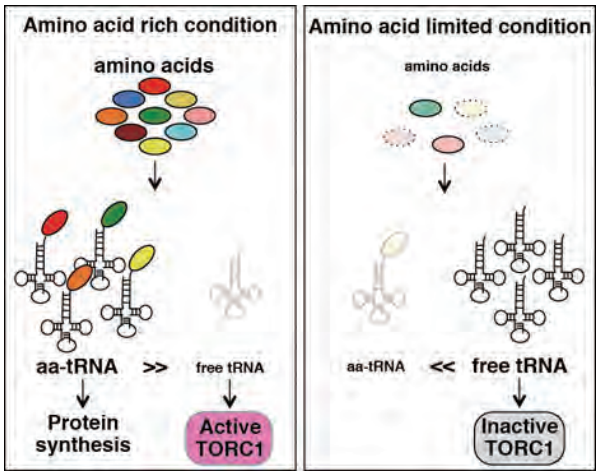

Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

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

We were consequently able to discover 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.

Publication List:

〔Original paper〕

● Takeda, E., Jin, N., Itakura, E., Kira, S., Kamada, Y., Weisman, L.S., Noda, T., and Matsuura, A. (2018). Vacuole-mediated selective regulation of TORC1-Sch9 signaling following oxidative stress. Mol. Biol. Cell *29*, 510-522.

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

● Baba, M., Tomonaga, S., Suzuki, M., Gen, M., Takeda, E., Matsuura, A., Kamada, Y., and Baba, N. A nuclear membrane-derived structure associated with Atg8 is involved in the sequestration of selective cargo, the Cvt complex, during autophagosome formation in yeast. Autophagy 2018 Oct 11.

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 (*e.g.* the mosquito's egg development neurosecretory hormone, the sea hare's egg-laying hormone, and the terrestrial isopod's androgenic gland hormone) 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*, thus 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, and acts 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 the 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 at Kyushu Univ., we are searching for reproductive hormones in echinoderms, including starfishes, brittle stars, sea urchins, sea cucumbers, and crinoids. The collaborating parties 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

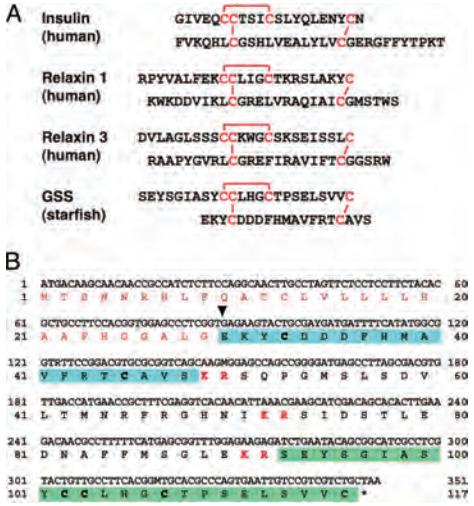

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

them, named cubifrin, which is an IWMGY-amide peptide, is in the sea cucumber *Aposticopus japonicus*. The others are in preparation for publication.

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. In particular, 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) to provide to collaborating researchers for biological assays.

III. Search for the lost mutants of female hormones E2 in *Oryzias latipes*

Sequence analysis by capillary sequencer was carried out from the tilling library of *O. latipes* in order to analyze the function of fish female reproductive hormone E2 as a part of our collaborative research. Upon the discovery of aromatase mutants, a detailed analysis was carried out using these strains.

Publication List:

〔Original paper〕

● 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. (2018). Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. *460*, 104-122.

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 a large impact on gene expression and evolution. To understand these genome dynamics in eukaryotes, especially in plants, we are analyzing 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. However a number of mutants displaying particular pigmentation patterns have been collected for this study. Because flower pigmentation patterns are easily observable, the molecular mechanisms underlying these phenomena provide useful model systems for investigating genome variability.

The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers. They are caused by a stable insertion of a transposable element into a gene for flower pigmentation. Furthermore, epigenetic mechanisms are thought to regulate thispigmentation (Figure 1). We are currently analyzing the detailed molecular mechanisms of these mutations.

Figure 1. The *duskish* mutant of *I. nil* shows variable flower phenotypes and produces variegated, fully pigmented, and pale grayish-purple flowers. It segregates offsprings that only show fully pigmented or pale grayish-purple flowers, and their phenotypes can be stably inherited by further generations.

II. Flower and seed pigmentation

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*. In line with this, we are conducting an international collaboration with researchers in the Netherlands and Switzerland to reveal the function of the ABC protein.

We also analyzed seed coat pigmentation by proanthocyanidin. Anthocyanin and proanthocyanidin biosynthesis pathways partially overlap. Mutant and genome analyses suggested that *I. nil* produces flavan-3-ols, the starter and extension units of proanthocyanidin, through an undiscovered biosynthesis pathway (Park *et al*., 2018).

III. BioResource of morning glories

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glories. In this project, we are collecting, maintaining and distributing standard and mutant lines for flower pigmentation, andDNA 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 related to it. Our collection include 240 lines and 160,000 DNA clones. The end sequences of the DNA clones can be viewed via the *I. nil* genome database (http://viewer.shigen.info/asagao/index.php).

Figure 2. New mutants of *I. nil* have been isolated from the large screening program that is a part of the activities conducted at NBRP. They appeared spontaneously from transposon active lines. (a) A variegated flower mutant resembling the *duskish* mutant (Figure 1). (b) A double flower mutant. (c) A mutant with increased number of shoot branches that is a hallmark of phytohormone (strigolactone) deficiency.

Publication List:

〔Original paper〕

- Park, K.I., Nitasaka, E., and Hoshino, A. (2018). Anthocyanin mutants of Japanese and common morning glories exhibit normal proanthocyanidin accumulation in seed coats. Plant Biotech. *35*, 259-266.

〔Review article〕

- Morita, Y., and Hoshino, A. (2018). Recent advances in flower color variation and patterning of Japanese morning glory and petunia. Breed. Sci. *68*, 128-138.

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*, in rice (*Oryza sativa L.*) is said to generate various transposon-insertion mutants because *nDart1* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1* 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.). All plants' genomes were isolated for identifying the insertion sites of *nDart1*.

I. *Large grain* (*Lgg*) mutation in rice

Seed size and number were controlled by various genes in the plants. It was reported that expression changes in high contribution genes for seed size, number and panicle shape resulted in a decrease of the total yield. A strategy for boosting rice yield based on molecular biology is to stack the finely tuned gene expressions. The *Lgg* mutant which was isolated from MK-1 plants bore slightly larger grains (Figure 1) as a dominant inheritance. Transposon-display identified the insertion site of *nDart1* in the *Lgg* mutant.

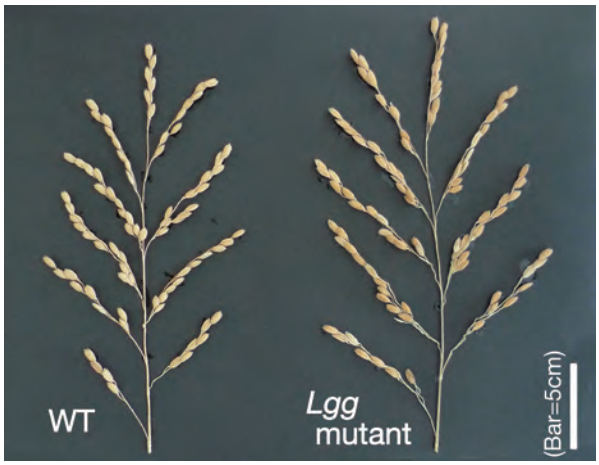

Figure 1. Phenotype of *Large grain* (*Lgg*). Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

The identified *LGG* gene shows similarity to RNA binding proteins. In the *Lgg* mutants, insertion of *nDart1* and genomic deletion was confirmed in a 5' untransrated region of the *LGG* gene. It was estimated that genomic deletion in *Lgg* mutants derived from microhomology in both the *nDart1* and *LGG*-genomic region. The expression of the *LGG* gene in *Lgg* mutants was lower than WT. *Lgg* mutation was essentially caused by the insertion of *nDart1*, knock-out mutant using genome editing (GE) and over-expressing (OE) lines were generated (Figure 2). GE lines showed large grain phenotypes that were the same as *Lgg* mutants. On the contrary, harvested seeds from the OE line were smaller than the WT and GE lines. To confirm that *Large grain* in *Lgg* mutants was caused by increasing the cell size or cell number or both, we developed a cross section method of rice seeds by using cryomicrotome (Chiou *et al*. 2018).

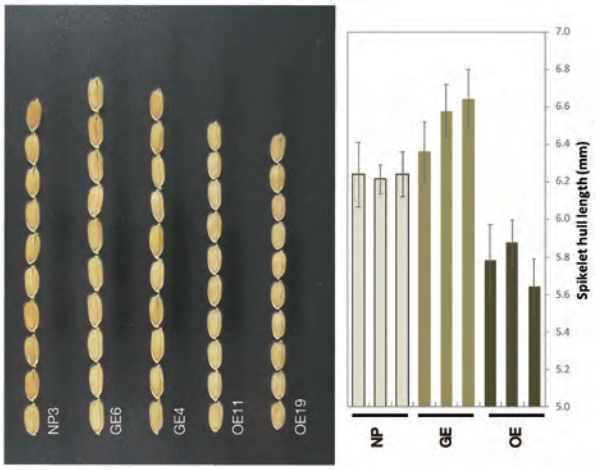

Figure 2. Phenotypes of wild type (NP), the genome-editing line (GE), and the overexpression line (OE). (Left)The spikelets. Bar = 5 mm. (Right) Spikelet hull length of NP, GE) and OE plants. Different letters indicate $P < 0.05$ by Fisher's Least Significant Difference test. $n = 10$. mean ± SD.

Publication List:

〔Original paper〕

● Chiou, W.Y., Tsugane, K., Kawamoto, T., and Maekawa, M. (2018). Easy sectioning of whole grain of rice using cryomicrotome. Breeding Science *68*, 381-384.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: *JOHZUKA, Katsuki*
Technical Staff: *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 decreasing chromome arm length, 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, which results in cell death. Chromosome condensation is mainly achieved by condensin, a hetero-pentameric protein complex, widely conserved across a variety of organisms ranging from yeast to humans. Despite its conservation and importance in chromosome dynamics, it is not fully understood how condensin works. Recent studies have revealed that condensin functions are not restricted to chromosome condensation and segregation during cell divisions, and is required for diverse DNA metabolism such as genome stability, transcriptional regulation, and cell differentiation.

Our aim is to understand the mechanism and regulation of chromosome condensation. To this end, we have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. Microscopic observation has 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 the recruitment of condensin to the RFB site. These proteins are known as components of the monopolin complex required for faithful segregation of homologous chromosomes during meiotic division I. During meiosis I, the monopolin complex re-localizes from rDNA repeat to the centromere and acts to ensure sister-chromatid co-orientation. Re-localization of Csm1/Lrs4 proteins suggested that re-localization of condensin from rDNA repeat to centromere had occurred. 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, functions as a cis-element for the 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 into an ectopic chromosome arm with an interval of 15kb distance in the cell with a 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 discovered 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 the 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 the internal regions of the 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.

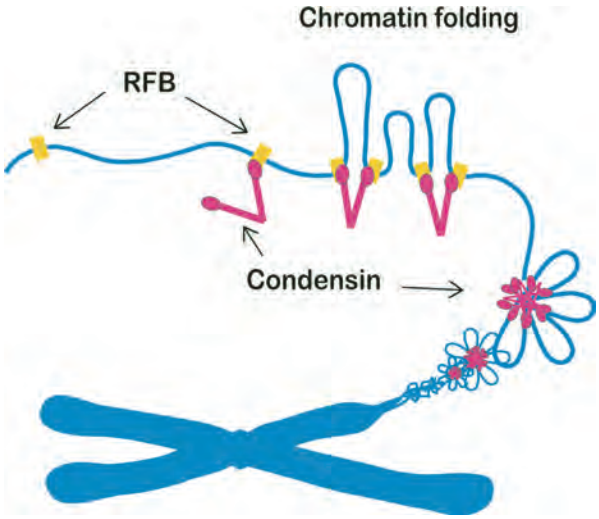

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

Specially Appointed Assistant Professor: *KATO, Kagayaki*
Technical Assistant: *HYODO, Miwa*

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 the integrated result of individual cell behaviors in response to spatio-temporally controlled mechanisms.

To better understand the programs underlying organ formation, it is necessary to quantitatively analyze individual cells' morphology and dynamics. However, it is difficult to do so due to the massive images generated by 4D microscopy and their ambiguity.

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

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in developing embryos is considered to be an important model for collective cell migrations. Drastic cell rearrangements lead to drastic structural changes in building 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 more 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 (Shinoda *et al*.).

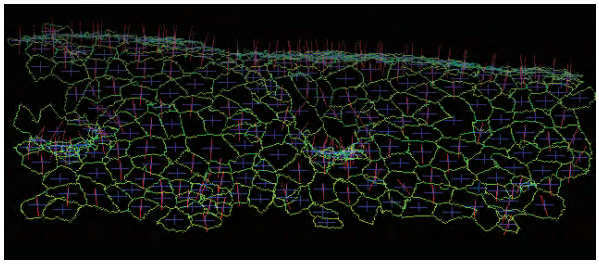

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

II. Image processing pipeline for 3D cell culture

To elucidate the relationship between mechanical forces and epithelial deformation, we developed an image processing pipeline for segmentation of nucleus within 3D culture of MDCK cells. This pipeline automated a segmentation/quantification process of a large number of images acquired by several experimental conditions for subsequent statistical analysis (Nishimura *et al*.).

III. Software for manual image quantification

Biologically significant imaging 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 have developed can easily visualize 4D imaging data and has made manual feature annotations easier (Figure 2). This application is freely available at our website (https://bioimageanalysis.jp/).

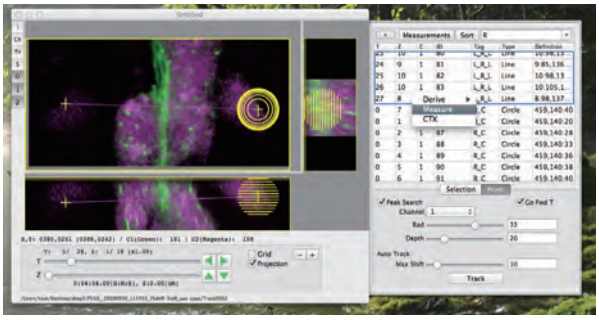

Figure 2. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

Publication List:

〔Original papers〕

- Nishimura, R., Kato, K., Fujiwara, S., Ohashi, K., and Mizuno, K. (2018). Solo and keratin filaments regulate epithelial tubule morphology. Cell Struct. Funct. *43*, 95-105.
- Shinoda, T,, Nagasaka, A., Inoue, Y., Higuchi, R., Minami, Y,. Kato, K., Suzuki, M., Kondo, T., Kawaue, T., Saito, K., Ueno, N., Fukazawa, Y., Nagayama, M., Miura, T., Adachi, T., and Miyata, T. (2018). Elasticity-based boosting of neuroepithelial nucleokinesis via indirect energy transfer from mother to daughter. PLoS Biol. *16*. e2004426.

LABORATORY OF BIOLOGICAL DIVERSITY†

KIMORI Group

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

Image processing methods significantly contribute to the visualization of biomedical targets acquired from a variety of imaging techniques, which include wide-field optical and electron microscopy, X-ray computed tomography, magnetic resonance imaging and mammography. However, quantitative interpretation of the wide range of complicated biomedical images poses many challenges for research. To counter these, 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. To this end, mathematical morphology is a nonlinear image processing method based on set theory, which 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. Contrast enhancement of structural details of lesion regions plays an especially important role in diagnostic imaging. It improves image quality and aids in clinical diagnosis. By using contrast enhancement methods, more accurate medical diagnoses can be expected. However, conventional image enhancement techniques also emphasize the noise and structure of various normal tissues other than regions containing legions.

In this study, a contrast enhancement approach based on a new type of mathematical morphology was 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 them 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 the enhancement of an abnormal region in the chest radiograph shown in Figure 1. The arrow in Figure 1(a) indicates the position of the abnormality (lung nodule). Figure 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.

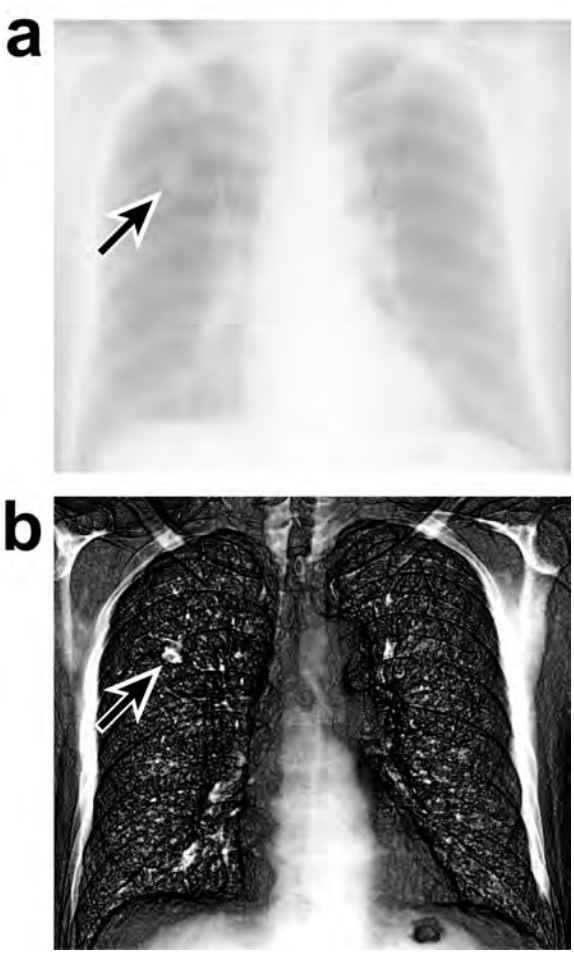

Figure 1. Contrast enhancement results for a chest x-ray image: (a) Original chest radiograph. (b) Contrast enhanced image by using the proposed method. The image was obtained from a standard digital image database of chest lung nodules and non-nodules (Shiraishi *et al*., Am. J. Roentgenol. *174*, 2000).

Publication List:

〔Original papers〕

- Aoyagi, Y., Hibi, T., Kimori, Y., Sawada, M., Kawakami, R., Sawamoto, K., and Nemoto, T. (2018). Heterogeneous distribution of doublecortin-expressing cells surrounding the rostral migratory stream in the juvenile mouse. J. Comp. Neurol. *526*, 2631-2646.
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†: This group was closed on 31 March, 2018.

DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

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

I. LHCSR1-dependent fluorescence quenching is mediated by excitation energy transfer from LHCII to photosystem I in *Chlamydomonas reinhardtii*

Photosynthetic organisms are frequently exposed to light intensities that surpass the photosynthetic electron transport capacity. Under these conditions, the excess absorbed energy can be transferred from excited chlorophyll in the triplet state (^{3}Chl*) to molecular O_2, which leads to the production of harmful reactive oxygen species. To avoid this photooxidative stress, photosynthetic organisms must respond to excess light in an appropriate manner. In the green alga *Chlamydomonas reinhardtii*, the fastest response to excess light is nonphotochemical quenching, a process that allows safe dissipation of the excess energy as heat. The two proteins, UV-inducible LHCSR1 and blue light-inducible LHCSR3, appear to be responsible for this function. While the LHCSR3 protein has been intensively studied, the role of LHCSR1 has only been partially elucidated. To investigate the molecular functions of LHCSR1 in *C. reinhardtii*, we performed biochemical and spectroscopic experiments and found that the protein mediates excitation energy transfer from light-harvesting complexes for Photosystem II (LHCII) to Photosystem I (PSI), rather than Photosystem II, at a low pH. This altered excitation transfer allows remarkable fluorescence quenching under high levels of light. Our findings suggest that there is a PSI-dependent photoprotection mechanism that is facilitated by LHCSR1 (Figure 1).

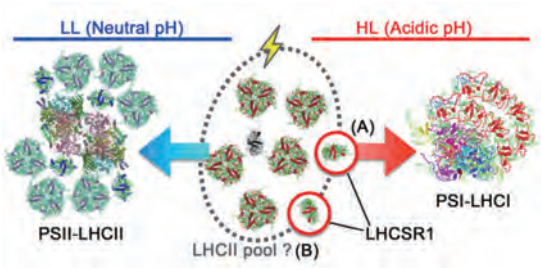

Figure 1. Tentative model of LHCSR1-dependent energy quenching in *C. reinhardtii*. The light captured by LHCIIs is transferred to the PSII–LHCII or PSI–LHCI supercomplexes under LL (representing neutral pH in the lumen, blue arrow) or HL (representing acidic pH in the lumen, red arrow), respectively. When the lumen is acidified, LHCSR1 (process A) mediates excitation energy transfer from LHCIIs in the pool to PSI-LHCI (this study) and/or (process B) triggers energy-dependent quenching in the LHCII pool. Icons surrounded by a red line represent LHCSR1. Kosuge *et al*. (2018) Proc. Natl. Acad. Sci. U. S. A.,115: 3722-3727.

II. Investigation on the thermodynamic dissociation kinetics of PSII supercomplexes to determine the binding strengths of LHCs

The PSII supercomplex splits water utilizing light energy, and is composed of a core dimer complex surrounded by LHCs. In green algae, the major LHCs which are LHCII trimers have thus far been categorized as either strongly, moderately, or loosely binding LHCII trimers based on their predicted binding to core complexes. However, the binding energies have been indirectly predicted based on the presence or absence of LHCII trimers in the PSII supercomplex under

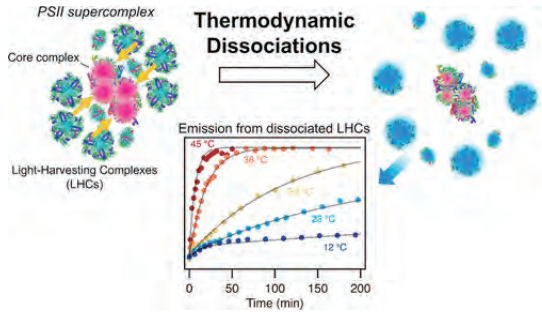

Figure 2. Scheme for thermodynamic dissociation kinetics of PSII supercomplexes. In case of intact PSII supercomplexes, fluorescence emission from LHCs is negligible because excitation energy transfer from LHCs to core complexes is sufficiently efficient. However, when LHCs are dissociated from the core complexes, fluorescence emission from LHCs are dramatically increased due to the lack of excitation energy transfer to the core complexes. Thermodynamic dissociation was observed by using this feature and analyzed to identify binding properties of PSII supercomplexes. Kim *et al*. (2018) J. Phys. Chem. B, 122: 1627-1630.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

electron microscopy and have not been determined experimentally. We investigated the binding of LHCII trimers by analyzing thermodynamic dissociation kinetics using isolated PSII supercomplexes (Figure 2). We then identified two activation energies for dissociation of LHCII trimers: 54 ± 19 and 134 ± 8 kJ/mol. This result indicated the types of intermolecular interactions between LHCII trimers and core complexes.

III. ROC75 is an attenuator for the circadian clock that controls LHCSR3 expression

In *Chlamydomonas reinhardtii*, LHCSR3 is required for the rapid protective response known as energy-dependent quenching (qE). Because the majority of photoacclimation analysis has been conducted under controlled laboratory conditions, physiological responses to natural environmental changes such as light/dark cycles have not been examined in detail. Regarding fitness in higher plants and microalgae, light-dark cycles represent a major Zeitgeber for synchronizing the circadian clock to multiple physiological responses, yet there is little consensus with respect to the clock response to high-intensity light in photosynthetic organisms. In a previous study, 105 circadian rhythm insertional mutants were isolated as rhythm of chloroplast (roc) mutants, including roc75 mutants. We performed transcript analysis of ROC75 in the pcry (plant-cryptochrome) and phot mutants and found that only the former accumulated lower levels of ROC75 mRNA, suggesting that the blue light photoreceptor pCRY positively regulates ROC75. However, the degradation of pCRY from excessive light exposure contributes to the prevention of ROC75 over-accumulation, which in turn facilitates the PHOT mediated main activation pathway for LHCSR3. Furthermore, LHCSR3 mRNA exhibited a circadian rhythm, though its basal expression level in the roc75 mutant was higher than that in WT. We therefore conclude that ROC75 acts as an attenuator of the circadian clock to control LHCSR3 expression with blue and red light as stimuli for attenuation.

IV. Green fluorescence from corals attracts symbiotic algae *Symbiodinium*

Reef-building corals form an obligate symbiotic relationship with dinoflagellates of the genus *Symbiodinium*. Symbiosis is established either through the inoculation of the developing oocyte, known as vertical transmission, or propagules are released free of symbionts that must therefore be acquired de novo from the environment, a process known as horizontal transmission. Over 70 % of corals undertake horizontal transmission. While the precise timing of symbiont acquisition in species performing horizontal transmission is unknown, both larvae and juveniles can take up a range of symbionts in the field. Uptake of symbionts by adult coral is also thought to occur, particularly following stress, such as bleaching. Given that corals are sessile for most of their life-history, with the exception of a comparatively brief larval stage, the coral host must rely on free living *Symbiodinium* to come to them. Most free-living *Symbiodinium* have a motile stage, during which they swim using two long flagella. The probability of an encounter between host and symbiont would be greatly increased if the host could attract these motile symbionts because of low *Symbiodinium* densities found on coral reefs.

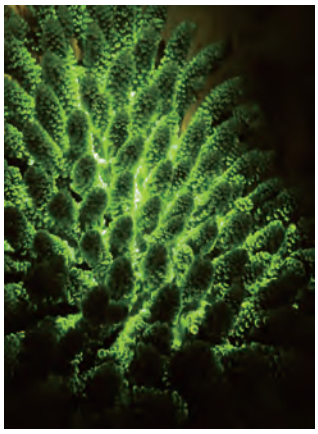

Figure 3. Emission of green fluorescence from corals under the exposure to blue light.

We focused on green fluorescence protein (GFP)-associated fluorescence, which is commonly seen in coral (Figure 3), and examined whether it attracts motile *Symbiodinium*. We first examined their phototaxis behavior and found that *Symbiodinium* shows positive and negative phototaxis mostly toward strong blue and weak green light, respectively. Attraction of *Symbiodinium* by green fluorescence was observed using both a live coral fragment and an artificial green-fluorescence dye but only under blue light *i.e.* the wavelength that induces green florescence. We also show that traps painted with a green fluorescence dye attracted *Symbiodinium* in the field. Our results revealed a novel biological signaling mechanism between the coral host and its potential symbionts (Aihara, Y., *et al*. Proc. Natl. Acad. Sci. U. S. A. *116*, 2118-2123).

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- Kim, E., Tokutsu, R., and Minagawa, J. (2018). Investigation on the thermodynamic dissociation kinetics of photosystem II supercomplexes to determine the binding strengths of light-harvesting complex. J. Phys. Chem. B, *122*, 1627-1630.
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- Okubo, N., Takahashi, S., and Nakano, Y. (2018). Microplastics disturb the anthozoan-algae symbiotic relationship. Mar. Pollut. Bull. *135*, 83-89.
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〔Original papers (E-publication ahead of print)〕

- Aihara, Y., Fujimura-Kamada, K., Yamasaki, T., and Minagawa, J. Algal photoprotection is regulated by the E3 ligase CUL4-DDB1^{DET1}. Nature Plants 2018 Dec 31.
- Toyoshima, M., Sakata, M., Ohnishi, K., Tokumaru, Y., Kato, Y., Tokutsu, R., Sakamoto, W., Minagawa, J., Matsuda, F., and Shimizu, H. Targeted proteome analysis of microalgae under high-light conditions by optimized protein extraction of photosynthetic organisms. J. Biosci. Bioeng. 2018 Sep 28.

DIVISION OF PLANT ENVIRONMENTAL RESPONSES

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Plant organs have the ability to sense various vectorial stimuli such as light, humidity, gravity, *etc*. and to reorient their growth direction so as to enhance their chances of survival and acclimatize to their environments. These responses in plant organs are referred to as tropisms. Gravitropism is one of major determinant for the direction of the growth angle of plant organs. In gravity sensing cells (statocytes), plastids that accumulate starch in high-densities relocate toward the direction of gravity. Amyloplasts relocationis used as signal to physically trigger biochemical signal transduction, which in turn leads to the regulation of the polar auxin transport necessary for change in the growth direction of plant organs.

The above points have raised the following important questions: 1) How is amyloplast relocation converted into biochemical signals? 2) How does the signal affect the directional plant growth? We aim to understand the detailed molecular mechanism of gravity signaling by applying a genetical and molecular biological approach using model plant *Arabidopsis thaliana*. We are currently focusing on the function of novel gravity signaling factor *LAZY* (*LZY*) genes and its interactors.

I. Analysis of molecular function *LZY* genes

To identify genes involved in gravity signaling in statocytes, we carried out transcriptome analysis using statocyte-deficient Arabidopsis mutants and found that several *LZY* genes are specifically expressed in the statocytes. We have shown that *LZY* genes are required for gravity signal transduction in statocytes following amyloplast relocation, thus determining the growth angle of plant organs (Figure 1). We are currently focusing on the analyses of the molecular function of LZY and the regulation of LZY protein levels.

1-1 *LZYs* are key gravity signaling factors in Arabidopsis roots and shoots

We have shown that the *lzy1 2 3* triple mutant exhibits greatly reduced gravitropism both in its primary roots and shoots. The differential auxin distribution in the organs, that is necessary for gravitoropic bending failed form in the mutant roots. Detailed observation indicated that development and behavior of amyloplasts in the triple mutant are normal. Furthermore, artificial expression of *LZY3* in statocytes clearly complemented the phenotype of gravitropism in the triple mutant. This experimental evidence strongly suggests that *LZYs* play an important role in gravity signaling after amyloplasts relocation.

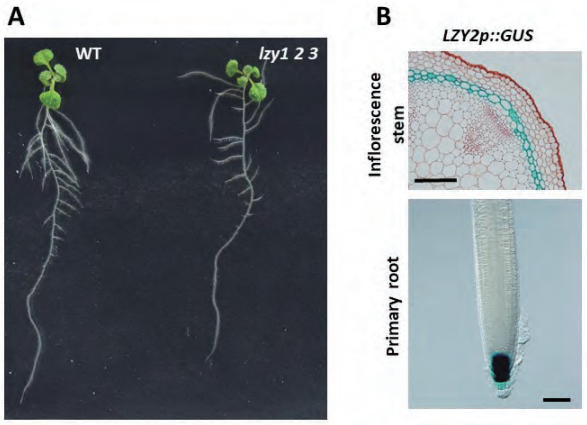

Figure 1. A, Phenotype of *lzy1 2 3* triple mutant. The mutant shows defect in growth angle of lateral roots. B, Expression analysis of *LZY2p:GUS*. Clear GUS staining are observed in endodermal cells in shoot and columella cells in root, respectively.

1-2 Subcellular localization of LZY3-mCherry in columella cells

We have found that a very low level of LZY proteins function within gravity signaling. Clear-see method barely visualized the fluorescence signal of LZY3-mCherry driven by native *LZY3* promoter in columella cells. Interestingly, LZY3-mCherry was localized on the plasma membrane at the bottom side of cells. The localization also appeared to change in response to the organ inclination. The plasma membrane localization of LZY3 was also confirmed by transient assay using Arabidopsis cultured cells (Figure 2). Analysis of amino-acid sequence of LZYs indicated no putative transmembrane domain. We are currently trying to reveal the mechanism of membrane localization of LZY3.

1-3 Protein level and tissue distribution of LZY3 affects growth angle of lateral roots

Under the control of the native LZY3 promoter, a feint *LZY3-mCherry* signal was detected in columella cells, indicating a very low expression level of LZY3 protein. To investigate the relationship between the expression level of LZY3 and gravitropic response, we generated a transgenic plant harboring LZY3-mCherry driven by the statocytes specific promoter combined with β-estradiol inducible system. We found that the protein level of LZY3-mCherry is able to be controlled in estradiol concentration dependent manner in columella cells. The proper induction of LZY-mCherry clearly complemented *LZYs* phenotype, whereas an excessive induction disturbed the direction of the growth angle in roots. Observations using a confocal microscope revealed that excessive LZY3-mCherry was localized on the plasma membrane uniformly in columella cells treated with a high concentration of β-estradiol. We found that the treatment of proteasome inhibitor MG132 significantly increased the LZY3-mCherry signal in the columella cells of plants carrying *LZY3p:LZY3-mCherry*. These results suggest that

the existence of proteasome-dependent regulatory mechanism in regulating the proper LZY3 protein level.

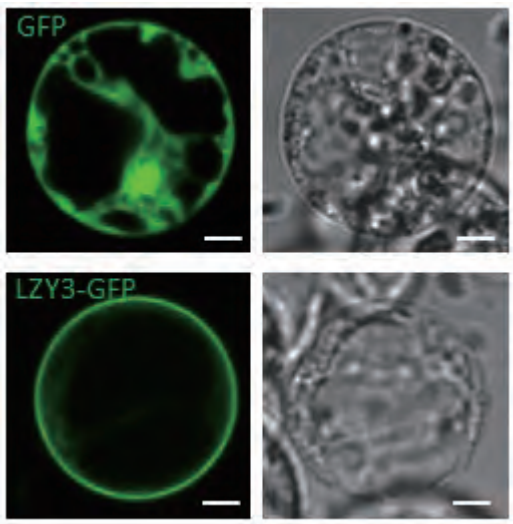

Figure 2. LZY3-GFP localization in protoplast of Arabidopsis cultured cells. Bar=10 μm

II. RCC1-like domain (RLD) proteins as LZY-interactor.

We have so far tried to identify LZYs interacted protein using yeast two-hybrid system and immunoprecipitation coupled with mass spectrometry in order to elucidate the molecular function of LZYs. We identified RLD family proteins as candidates. The *RLD* family is composed of eight genes in Arabidopsis and contain PH domain, RCC1-like motif repeat, FYVE domain and BRX domain. Further analysis revealed that LZY C-terminal 14 amino acids interact with BRX domain in RLDs. Also, LZYs seems to recruit RLDs to the plasma membrane through their mutual interaction. Among RLD family genes, *RLD1* and *RLD4* contribute gravity signaling in columella cells. Because *rld1, 2, 3, 4* quadruple mutant seedlings show severe morphological defects, which resemble those of plant ARF-GEF *gnom* mutant, *RLDs* are possibly involved in membrane trafficking. We are analyzing the biochemical activity of RLD proteins, as well as characterizing the molecular function of *RLD5-8*.

Publication List:

〔Original paper〕

- Yamamoto, T., Yoshida, Y., Nakajima, K., Tominaga, M., Gyohda, A., Suzuki, H., Okamoto, T., Nishimura, T., Yokotani, N., Minami, E., Nishizawa, Y., Miyamoto, K., Yamane, H., Okada, K., and Koshiba, T. (2018). Expression of RSOsPR10 in rice roots is antagonistically regulated by jasmonate/ethylene and salicylic acid via the activator OsERF87 and the repressor OsWRKY76, respectively. Plant Direct *2*, e00049.

DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

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Animals living outside the tropics adapt various aspects of their physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize the survival of their offspring in temperate zones. The differing approaches that animals use as seasonal cues to recognize changes in day length and temperature 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 temperature

It is well established that temperature changes are important in seasonal time measurement. However, it remains unknown how animals measure temperature changes regarding seasonal time measurement. It has been reported that medaka populations that are caught at higher latitudes have more sophisticated responses to seasonal changes. To uncover the underlying mechanism of seasonal time measurement, we are currently performing a forward genetic analysis among medaka populations collected from various latitudes across Japan.

1-1 Variation in critical temperatures in line with latitudinal differences in medaka fish

To perform a forward genetic analysis, we have obtained 5 populations including wild populations and closed colonies from all over Japan. We have examined the effects of changing temperatures to determine the critical temperature that will cause seasonal responses in the gonad. In winter, fish were subjected to 14, 16, 18, and 20 degree temperatures over a long day length. Gonadal development was examined to identify the critical temperature.

As a result, we found differences in the critical temperature among medaka populations (Figure 1). It was found that medaka from higher latitudes required higher temperatures while those from lower latitudes required lower temperatures.

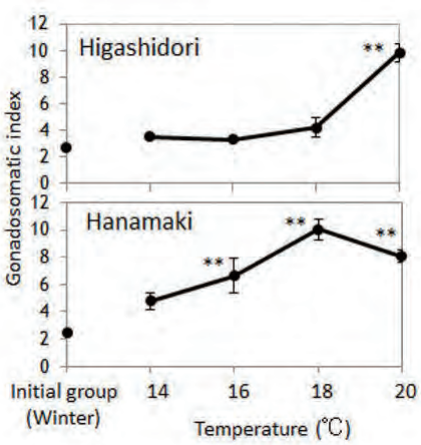

Figure 1. Differences in critical temperature.

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

To identify the genes regulating critical temperature, quantitative trait loci (QTL) analysis was conducted using F_2 medaka derived from crosses between Northern and Southern Japanese populations. We identified significant QTL on chromosome 12 using Restriction-site Associated DNA (RAD) markers. We have also performed whole genome re-sequencing using various medaka strains that show different critical temperatures, and identified potential candidate genes that define the critical temperature.

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 separates seasonal breeders from 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 the medaka from lower latitudes' gonads did not regress even under short day conditions. Accordingly, we then examined the responses to short day conditions using 20 populations derived from various latitudes. As a result, we found that populations from higher latitudes showed gonadal regression, while populations from lower latitudes did not regress their gonads.

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 the brain of the medaka fish to understand the underlying mechanism of seasonal adaptation.

3-1. Identification of photoperiodically-regulated transcripts

During the breeding season, animals show stress responses, such as self-protective and escape behaviors, when confronted with potentially dangerous situations such as predation, interspecific competition, and harsh weather. These behaviors are critical for animals to survive changing environments. Photoperiod is the most reliable indicator of the forthcoming season, and seasonal regulation of various physiological and behavioral processes by photoperiod has been known for decades. However, the molecular mechanisms underlying seasonal adaptive strategies are not well understood. Medaka fish serve as an excellent model to study the mechanism of seasonal adaptation, because of their highly sophisticated seasonal responses and the recent availability of genomic information and genome-editing tools. Interestingly, we recently demonstrated that dynamic seasonal changes in color perception alters breeding behavior in medaka. To further understand the molecular basis of seasonal adaptation in medaka, we performed genome-wide transcriptome analysis of their brains during the transition from short day (SD) to long day (LD) conditions and identified several photoperiodically-regulated genes and signaling pathways (Figure 2).

3-2. Photoperiodically-regulated, cycling transcript was a lncRNA

Expression of one transcript named *olvl28m13* was induced and was strongly rhythmic only under long day conditions (Figure 2). Notably, the timing of its induction was much earlier than nearly all up-regulated genes.

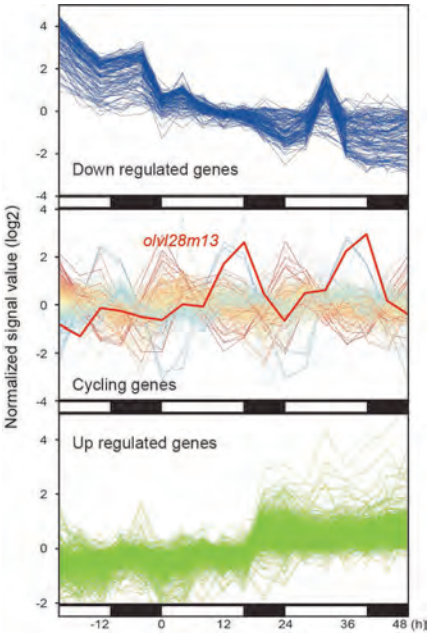

Figure 2. Transcriptional landscape during the transition from short day to long day.

To examine *olvl28m13* further, we next performed strand-specific RNA-seq analysis and confirmed higher expression under LD than SD conditions (Figure 3). This RNA was transcribed from the intronic region of the *LPIN2* gene. We then performed ribosomal profiling analysis (Ribo-seq) to determine the protein-coding potential of this transcript. The result of Ribo-seq indicated that the transcript *olvl28m13* is not associated with ribosomes and most likely not translated into protein. To examine functional significance of this lncRNA, we generated KO medaka using the CRISPR/Cas9 system. Behavioral analysis of the KO fish demonstrated that this lncRNA affects self-protective behaviors, suggesting that lncRNA modulates adaptive behaviors to seasonal environmental changes.

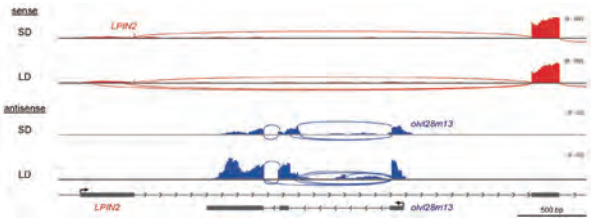

Figure 3. Strand specific RNA-seq identified *olvl28m13* in the intronic region of the *LPIN2* gene.

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〔Original papers〕

- Ota, W., Nakane, Y., Hatter, S., and Yoshimura, T. (2018). Impaired circadian photoentrainement in Opn5-null mice. iScience *6*, 299-305.
- Tamai, T.K., Nakane. Y., Ota, W., Kobayashi, A., Ishiguro, M., Kadofusa, N., Ikegami, K., Yagita, K., Shigeyoshi, Y., Sudo, M., Nishiwaki-Ohkawa, T., Sato, A., and Yoshimura, T. (2018). Identification of circadian clock modulators from existing drugs. EMBO Mol. Med. *10*, e8724.

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- Nakane, Y., and Yoshimura, T. (2018). Seasonal reproduction: Photoperiodism, Birds. In Encyclopedia of Reproduction 2nd Edition, M.K. Skinner, ed. (Academic Press Inc.) pp. 409-414.
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LABORATORY OF GENOME INFORMATICS

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The accumulation of biological data has recently been accelerated by various high-throughput "omics" technologies including genomics, transcriptomics, and proteomics. The field of genome informatics is aimed at utilizing this data, or finding the principles behind it, to understand complex living systems by integrating the data with current biological knowledge via the use of 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 interpret 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 by comparing 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, 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. By the application 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 viewing modes to display the entire picture of each ortholog table. For some closely related taxa, MBGD provides conserved synteny information calculated using the CoreAligner program. MBGD additionally provides MyMBGD mode, which allows users to add their own genomes to MBGD.

This year, we released the new version of MBGD, which contains 6318 genomes, including 5861 bacteria, 254 archaea, and 203 eukaryota. In addition to updating the database, we modified the protocol to construct ortholog table, which will be described in the next section.

II. Hierarchical strategy for creating ortholog tables

MBGD previously calculated all-against-all similarities among the stored genomes and created two types of ortholog tables independently: 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).

The problem with this approach is twofold. Firstly, rapid accumulation of the genomic data from the same or closely related species substantially expands the size of all-against-all similarity data, while the increased net amount of information (*i.e.* the size of gene repertoire) is more limited. Secondly, the standard ortholog table contains only genes that are contained in the representative genomes, and thus a considerable amount of information may be lost from the standard ortholog table, if one were to consider within-species and within-genus genomic diversity.

To address these problems, we developed a stepwise protocol to construct orthologous relationships. First, for each species having at least two genomes, all-against-all similarities among the genomes belonging to that species are calculated and a within-species ortholog table is created. The species-level pan-genome is then created by picking one representative gene from each orthologous group. Next, for each genus having at least two species, all-against-all similarities among the species-level pan-genomes and a within-genus ortholog table is created. The genus-level pan-genome is then created by picking one representative gene from each orthologous group. Finally, all-against-all similarities among the genus-level pan-genomes are calculated and the standard ortholog table covering the entire taxonomic range is created. To calculate within-species or within-genus all-against-all similarities, we used a faster but less sensitive similarity search program, UBLAST. In this way, we can reduce the computation time required for all-against-all similarities.

An example of a hierarchical ortholog group is shown below (Figure 1), where an ortholog group containing Shiga toxins is shown. Analysis of such sporadically distributed genes was often not possible in the previous version of MBGD, because such an ortholog group was often not contained in the standard ortholog table.

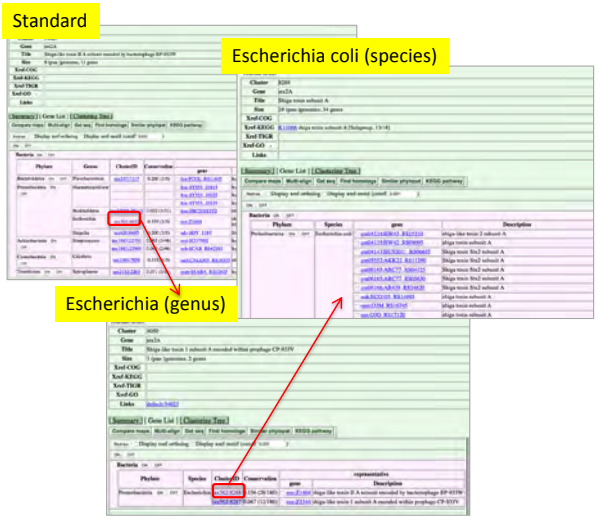

Figure 1. An example of a hierarchical ortholog group. Shown is the ortholog group containing Shiga-like toxins subunit A.

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 allows us to simultaneously compare many genomes. This 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. In particular, this data is considered to be suitable for analyzing domain fusion events that have occurred during evolution. By analyzing the domain-level ortholog grouping data combined with taxonomic and functional information, we are now 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 large-scale ortholog tables. The ortholog table viewer is a spreadsheet like viewer that can display an 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 performed 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 are continuing to develop the system and apply it to various genome comparison studies as part of various collaborative research projects (including *H. pylori* genome comparison described in Section VI below). In addition to microbial genome comparison, we are trying to apply RECOG to the 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 integrating knowledge about various organisms through comparative analysis. We have constructed an ortholog database using Semantic Web technology, and are aiming to integrate 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 auspices of the National Bioscience Database Center.

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. As we have already developed a procedure (coreAligner) to define the core genome as the genes conserved among the genomes of the given species, characterizing the remaining part of the genomes (non-core genomes) should be important for understanding the species’ diversity. To this end, we developed a method (FindMobile) to define mobility of genes against the reference coordinate determined by the core genome alignment, and classified each non-core gene into mobility classes. Combining this with a naive clustering procedure on the basis of phylogenetic pattern similarity and chromosomal proximity implemented in RECOG, we were able to identify genomic island candidates among the genomes of 30 *Helicobacter pylori* strains. We are now trying to generalize this approach to identify genomic islands in various bacterial species.

Publication List:

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

- Uchiyama, I., Mihara, M., Nishide, H. , Chiba, H., and Kato, M. MBGD update 2018: microbial genome database based on hierarchical orhtology relations covering closely related and distantly related comparisons. Nucleic Acids Res. 2018 Nov 20.

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 this 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, remain controversial, with several models being proposed, and the involvement of Ca^{2+} being suggested.

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

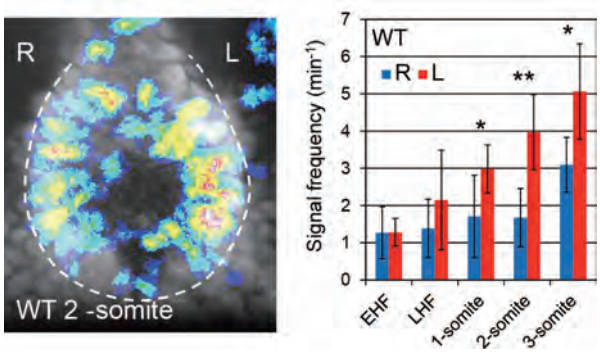

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

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

II. Development of light-sheet microscopy

Light-sheet microscopy has become popular during this decade for benefits such as low photodamage and fast image acquisition. We are now running two light-sheet microscopes, one commercial and the other self-made, and are maintaining them for collaborations and our own research interest (this being 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 greater speed and the exclusion of mechanical vibrations that disturb continuous 4D imaging of floating or loosely held specimens.

Our light-sheet microscopes are available 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*.

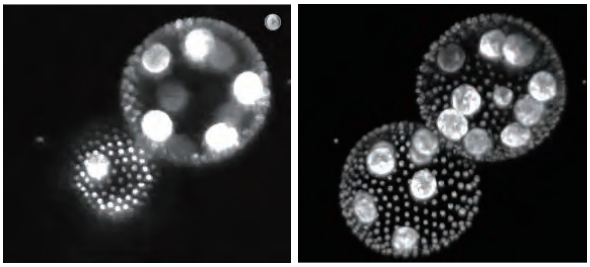

Figure 2. Images of floating volvox taken by ezDSLM with ETL. Left: Single optical section. Right: Maximum intensity projection.

Publication List:

〔Original papers〕

- Nishigami, Y., Ohmura, T., Taniguchi, A., Nonaka, S., Manabe, J., Ishikawa, T., and Ichikawa, M. (2018). Influence of cellular shape on sliding behavior of ciliates. Commun. Integr. Biol. *11*, e1506666.
- Ohmura, T., Nishigami, Y., Taniguchi, A., Nonaka, S., Manabe, J., Ishikawa, T., and Ichikawa, M. (2018). Simple mechanosense and response of cilia motion reveal the intrinsic habits of ciliates. Proc. Natl. Acad. Sci. USA *115*, 3231-3236.
- Okimura, C., Taniguchi, A., Nonaka, S., and Iwadate, Y. (2018). Rotation of stress fibers as a single wheel in migrating fish keratocytes. Sci. Rep. *8*, 10615.

LABORATORY OF NUCLEAR DYNAMICS

Specially Appointed Associate Professor
MIYANARI, Yusuke

JSPS Postdoctoral Fellow: *KURIHARA, Misuzu*
Postdoctoral Fellow: *KURIHARA, Misuzu**
SOKENDAI Graduate Student: *ISHII, Satoko*
Technical Assistant: *SANBO, Chiaki*
TAGAWA, Ayako
Secretary: *HACHISUKA, Midori*

A fundamental challenge in biology is the one of understanding the mechanisms underlying cell-fate decisions. Genomic reprogramming after mammalian fertilization reverts terminally differentiated gametes into toti- or pluripotent states when starting 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 role of chromatin dynamics in cell lineage-allocation by deciphering the molecular mechanisms underlying the remodeling of nuclear organization and their effects on developmental gene expression, using mouse embryos and embryonic stem (ES) cells as model systems.

I. Epigenetic reprogramming in early mouse embryos

Genomic reprogramming reverts fully differentiated cells to a totipotent state so they can start a new developmental program. In early mouse embryos, 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.

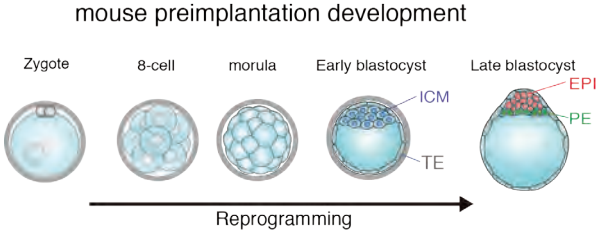

Figure 1. Lineage allocation in mouse preimplantation development

II. Remodeling of nuclear architecture in development

Chromatin is organized in a non-random fashion within three-dimensional nuclear space. During developmental processes, the nuclear architecture is dramatically reconstructed, resulting in the 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 the 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 decisions remain largely unknown.

III. Chromatin structure

Spatiotemporal organization of genomic DNA within the nucleus has been suggested as an emerging key player to regulate gene expression. The developmental program accompanies nuclear remodeling, resulting in construction of nuclear architecture specific to the cell type. 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 decisions remains largely unexplored.

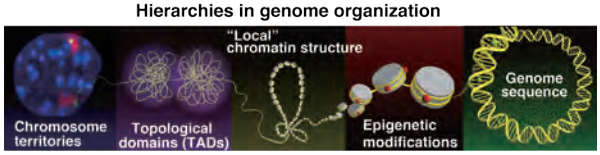

Figure 2. Hierarchical chromatin structure

IV. Approach

We have developed a powerful imaging technology called 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 many robust applications, which will in turn be integrated into our study to manipulate several genome functions. Based on this new technological development, we aim to understand the biological role of chromatin dynamics in cell-fate decision.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY

Specially Appointed Associate Professor
KAWADE, Kensuke

Adjunct Professor (BIO-NEXT Project, ExCELLS): *TSUKAYA, Hirokazu*
Postdoctoral Fellow: *NOZAKI, Mamoru*
SOKENDAI Graduate Student: *ST CLAIR, Griffin*
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 an instructive role in developmental processes. This 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 largely remains unclear how this interaction is established in multicellular organisms. We aim to reveal as-yet-unknown relationships between developmental and metabolic processes, and their biological meaning, by elucidating molecular mechanisms in the system. To address this, we use a 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 the hidden relationships between development and metabolism, we performed quantitative phenome screening using *A. thaliana* mutants of orphan cytochrome P450 genes. As a result of this screening, we discovered the *cyp77a4* mutant, which exhibits irregular embryonic patterning as evidenced by its developmental defects, such as a cup-shaped cotyledon morphology (Figure 1). Although CYP77A4 is the first cytochrome P450 reported that is able to catalyze the epoxidation of unsaturated fatty acids in plants, its function in development is 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 found that *CYP77A4* and *CYP77A6* (the phylogenetically closest gene to *CYP77A4*) are functionally independent. Based on these findings, we propose that the metabolic requirement for polarity establishment via auxin dynamics differs 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 new developmental function of the epoxidase (Kawade *et al*., 2018).

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. The role of the 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 *an3* mutants. We found that AN3 regulates a transcriptional network for oxygen homeostasis. Our metabolic profiling 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 the argument surrounding cell proliferation and oxidative stress, in plant development.

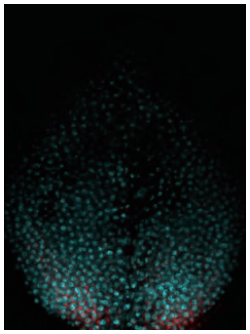

Figure 2. A merged image of the leaf primordia (around 200-μm length) expressing genetically engineered mobile or immobile AN3 (cyan or red, respectively). The distribution of the mobile AN3 gradually spreads along the leaf proximal-to-distal axis (from down to top), which is broader than that of the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

Publication List:

〔Original papers〕

● Ferjani, A., Kawade, K., Asaoka, M., Oikawa, A., Okada, T., Mochizuki, A., Maeshima, M., Hirai, M.Y., Saito, K., and Tsukaya, H. (2018). Pyrophosphate inhibits gluconeogenesis by restricting UDP-glucose formation *in vivo*. Sci. Rep. *8*, 14696.
● Kawade, K., Li, Y., Koga, H., Sawada, Y., Okamoto, M., Kuwahara, A., Tsukaya, H., and Hirai, M.Y. (2018). The cytochrome P450 CYP77A4 is involved in auxin-mediated patterning of the *Arabidopsis thaliana* embryo. Development *145*, dev168369.

ASTROBIOLOGY CENTER

Specially Appointed Associate Professor
TAKIZAWA, Kenji

The purpose of the Astrobiology Center (ABC) is to search for a so called 'second Earth' and extraterrestrial life. Researchers at the main office in Tokyo are looking for habitable planets around the nearest stars, developing observational instruments, and predicting bio-signatures via computational analyses, whereas I am performing biological experiments at the NIBB office to understand photosynthesis on Earth, and predict the nature of photosynthesis on what may be the 'second Earth'.

I. Predicting photosynthetic apparatus of "Alien" plants on habitable planets around M-dwarfs

M-dwarfs are stars that are most abundant when compared to other types, and are places where it is relatively easier to search for habitable planets. While the sun strongly radiates visible light, M-dwarfs are brighter in their near-infrared (NIR) region. If alien plants around M-dwarfs use NIR for photosynthesis, light reflection patterns and the amount of oxygen that is produced will be different for plants which use visible light. We estimated the light conditions on habitable planets, and discovered that light conditions in water were similar to the oceans found on earth since NIR cannot penetrate the many meters of water within them. Photosynthesis like that found on Earth (Earth type synthesis) which utilizes visible light to excite two photochemical reaction centers, could be a universal mechanism used by photothrophs which originate underwater. NIR radiation is stronger around the water surface, but its intensity can change drastically and thus it is difficult to excite two reaction centers evenly. NIR-using plants may evolve only after they have adapted to the land surface where strong and stable NIR radiation is supplied (Figure 1).

There are several hypothetical mechanisms that enable photosynthesis using NIR, but only a few of them can evolve from Earth-type photosynthesis. 'Two-color' reactions that use NIR at one reaction center and use visible light at the other may be a transitional mechanism. Our research involves investigating which mechanisms can be plausibly achieved by minor modifications of Earth-type photosynthesis.

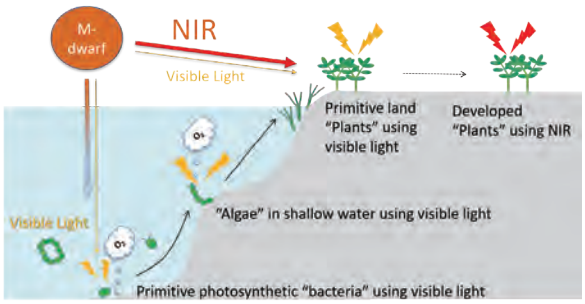

Figure 1. Hypothetical evolution scheme of photosynthetic organisms on habitable planets around M-stars.

II. In-situ assessment of photosynthesis in "Terrestrial" plants

Photosynthetic research has mostly progressed by laboratory experiments using controlled radiation. Conversely, adaptation mechanisms utilized in natural fluctuating light conditions should be investigated in the field. We provided a field survey of photosynthesis via the use of portable spectrophotometers (MultispeQ, Michigan State Univ). Sun plants that had adapted to strong visible light showed higher linear electron flow (LEF) capacity in maximizing productivity (Figure 2). Shade plants that had adapted to weaker light showed lower LEF and relatively high cyclic electron flow (CEF), thus suggesting that NIR radiation enhanced the adaptation mechanism for fluctuating light. If shade plants can use NIR for LEF, photosynthetic productivity will be increased, but it could be more susceptible to photodamage under fluctuating light conditions because of suppressed CEF.

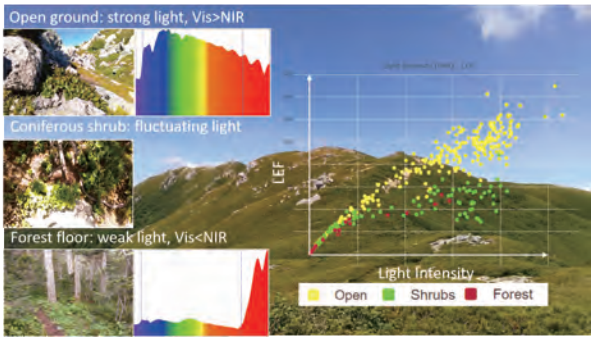

Figure 2. Field survey of photosynthesis of sun/shade plants at Central Alps, Japan (≈2,700 MASL). Sun plants growing on open ground shows higher LEF capacity, than shade plants on shrubs or forest floor.

III. Promoting interdisciplinary cooperation in the field of Astrobiology

ABC is promoting interdisciplinary research projects to cultivate the new field of astrobiology. Furthermore, the branch office at NIBB plays a role in introducing Astrobiology to young biologists via study meetings and seminars (Figure 3)."Biology" should be an equal partner with "Astronomy" in this endeavor. However, for the time being, contributions from biology have been insufficient.

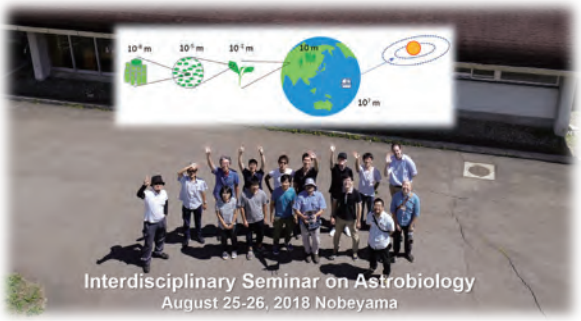

Figure 3. Interdisciplinary Seminar on Astrobiology. Young scientists studying in different fields (astronomy, biology, earth science, computational chemistry, and agriculture) got together for discussing about life on Earth and beyond.

NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities were launched in 2010 to support basic biological research at NIBB. They consist of three facilities that develop and provide state-of-the-art technologies aimed at increasing the understanding of biological functions through the application of functional genomics, bioimaging, and bioinformatics. The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among NIBB researchers 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 the promotion of DNA and protein studies. The facility maintains a wide array of core research equipment, ranging from standard machinery (*e.g.* ultracentrifuges) to cutting edge tools (*e.g.* next generation DNA sequencers), which amount to 90 instruments in total. The facility is dedicated to fostering collaborations with researchers both at NIBB and other academic institutions worldwide through the provision of 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 conducted a large scale renovation of the Functional Genomics Facility building, and as part of this, the Visitors Lab and the Visitors Office were re-designed so that visiting scientists can work more effectively during their stay. In 2018, approximately 200 researchers came to use our facility and developed active collaborations, which consequently resulted in 20 co-authored papers published.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming modern biology thanks to ultra-high-throughput DNA sequencing. Utilizing HiSeq1500, NextSeq and MiSeq (Illumina), PacBio RS II and Sequel (PacificBio Sciences), and MinION and GridION (Oxford Nanopore Technologies), the Functional Genomics Facility is committed to joint research aimed at exploring new yet otherwise inaccessible fields in basic biology.

During 2018, we carried out 67 NGS projects in collaboration with researchers from academic institutions throughout the world. These projects cover a wide range of species (bacteria, animals, plants, and fungi) including both model and non-model organisms, and various other applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

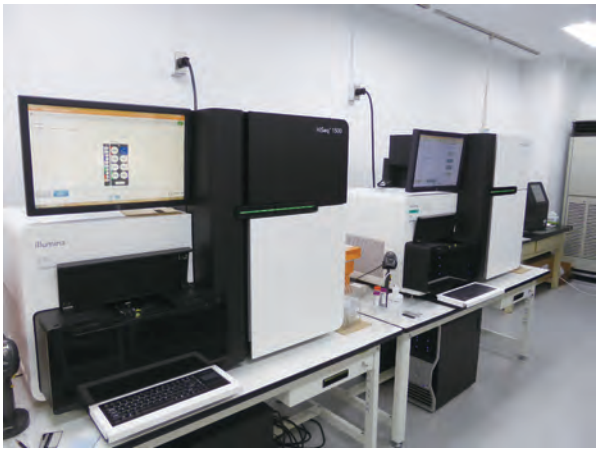

Figure 1. Next-generation sequencer

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2018, we analyzed approximately 1000 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)

Figure 2. Triple TOF LC/MS/MS System

Other analytical instruments (excerpts)

- Cell sorter (SONY SH800)
- Bioimaging analyzer (Fujifilm LAS 3000 mini; GE FLA9000)

- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500)
- Ultracentrifuge (Beckman XL-80XP *etc*.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1)

Publication List on Cooperation:

〔Original papers〕

- Ando, T., Matsuda, T., Goto, K., Hara, K., Ito, A., Hirata, J., Yatomi, J., Kajitani, R., Okuno, M., Yamaguchi, K., Kobayashi, M., Takano, T., Minakuchi, Y., Seki, M., Suzuki, Y., Yano, K., Itoh, T., Shigenobu, S., Toyoda, A., and Niimi, T. (2018). Repeated inversions within a *pannier* intron drive diversification of intraspecific colour patterns of ladybird beetles. Nat. Commun. *9*, 3843.
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〔Original paper (E-publication ahead of print)〕

- Matsunami, M., Nozawa, M., Suzuki, R., Toga, K., Masuoka, Y., Yamaguchi, K., Maekawa, K., Shigenobu, S., and Miura, T. Caste-specific microRNA expression in termites: insights into soldier differentiation. Insect Mol. Biol. 2018 Oct 16.
- Yoshida, K., Ishikawa, A., Toyoda, A., Shigenobu, S., Fujiyama, A., and Kitano, J. Functional divergence of a heterochromatin-binding protein during stickleback speciation. Mol. Ecol. 2018 Aug 17.

● *Research activity by S. Shigenobu*

Specially Appointed Associate Professor:	*SHIGENOBU, Shuji*
NIBB Research Fellow:	*OGAWA, Kota**
SOKENDAI Graduate Student:	*YORIMOTO, Shunta*
Visiting Scientist:	*CHUNG, Chen-yo*
	OGAWA, Kota
Technical Assistant:	*SUZUKI, Miyuzu*

Symbiogenomics

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

Every creature on 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 that are indigestible by the host alone. Despite 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 known as "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.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

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

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, which are specialized cells for harboring said bacteria. This 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 these two organisms. The genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. 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 in obtaining beneficial symbionts. Lineage-specific gene duplications have occurred in genes over 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 the 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 coinciding 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. Furthermore, some BCRs showed 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〕

- Nikoh, N., Tsuchida, T., Maeda, T., Yamaguchi, K., Shigenobu, S., Koga, R., and Fukatsu, T. (2018). Genomic insight into symbiosis-induced insect color change by a facultative bacterial endosymbiont, "*Candidatus* Rickettsiella viridis." MBio *9*, e00890-18.
- Suzuki, M., Hayashi, T., Inoue, T., Agata, K., Hirayama, M., Suzuki, M., Shigenobu, S., Takeuchi, T., Yamamoto, T., and Suzuki, K.-I.T. (2018). Cas9 ribonucleoprotein complex allows direct and rapid analysis of coding and noncoding regions of target genes in *Pleurodeles waltl* development and regeneration. Dev. Biol. *443*, 127–136.

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, under the guidance of Dr. Kamei, also provides technical support through the management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers (refer to the Collaborative Research Group Research Enhancement Strategy Office section for more information). Among the equipment available are advanced biological microscopes, and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism and is capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve, thus allowing exposure to strong monochromatic light. The facility's microscopes, which include cutting edge devices such as confocal and multi-photon excitation microscopes, are an indispensable part of core and collaborative projects conducted by both internal and external researchers.

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.

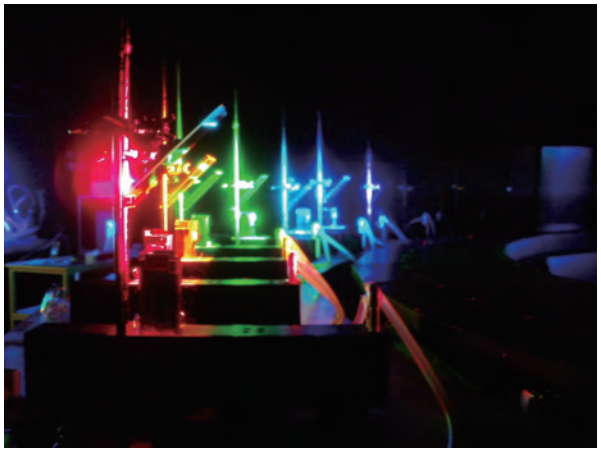


Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously to samples stored in cooling chambers.

In addition to the other action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted since it's establishment, 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.

Microscopes

This facility also provides bioimaging machinery, such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Leica TCS-SP8, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD/ CMOS cameras), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced laser microscopes boasting specialized, cutting edge technology (Light-sheet Microscope and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO), which can be utilized by researchers within NIBB, as well as collaborative guest researchers. We began two new types of Collaborative Research Programs from 2016. One is a new category of the NIBB Collaborative Research for Integrative Bioimaging program using machinery and bioimage processing/analysis techniques, and the other is the Advanced Bioimaging Support Program (ABiS) which operates under the framework 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 tissues by illuminating specimens 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 Collaborative Research Program projects for Integrative Bioimaging. The IR-LEGO, developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST), can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (details are provided in the next section). The IR-LEGO was also used for about 10 Collaborative Research projects, including applications for animals and plants.

Workshop, symposium and training course

In 2018 we held the 5th biological image processing training course with Drs. Kagayaki Kato, Shigenori Nonaka, Takashi Murata and Hiroshi Koyama (p. 104). 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 12th NIBB Bioimaging Forum focused on artificial intelligence (AI) for imaging field (p. 103). In addition, we held training courses focused on medaka, *Xenopus* and a new emerging model animal, Iberian ribbed newt, for domestic and also international participants (p. 101).

Publication List on Cooperation

〔Original papers (Selected)〕

- Hamada, T., Yako, M., Minegishi, M., Sato, M., Kamei, Y., Yanagawa, Y., Toyooka, K., Watanabe, Y., and Hara-Nishimura, I (2018). Stress granule formation is induced by a threshold temperature rather than a temperature difference in Arabidopsis. J. Cell Sci. *131*, jcs216051.
- Hasugata, R., Hayashi, S., Kawasumi-Kita, A., Sakamoto, J., Kamei, Y., and Yokoyama, H. (2018). Infrared laser-mediated single-cell-level gene induction in the regenerating tail of *Xenopus laevis* tadpoles. Cold Spring Harb. Protoc. *2018(12)*:pdb.prot101014.
- Kamijo, M., Kawamura, M. and Fukamachi, S. (2018). Loss of red opsin genes relaxes sexual isolation between skin-colour variants of medaka. Behav. Processes *150*, 25-28.
- Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T., and Hasebe, M. (2018). Physcomitrella MADS-box genes regulate water supply and sperm movement for fertilization. Nature Plants *4*, 36-45.
- Mano, S., Nishihama, R., Ishida, S., Hikino, K., Kondo, M., Nishimura, M., Yamato, K. T., Kohchi, T., and Nakagawa, T. (2018). Novel gateway binary vectors for rapid tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha*. PLoS ONE *13*, e0204964.
- Matsuo, M., Yoriko Ando, Y., Kamei, Y., and Fukamachi, S. (2018). A semi-automatic and quantitative method to evaluate behavioral photosensitivity in animals based on the optomotor response (OMR). Biology Open *7*, bio033175.
- Nagao,Y., Takada, H., Miyadai, M., Adachi, T., Seki, R., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Hibi, M., Kelsh, R.N., and Hashimoto, H. (2018). Distinct interactions of Sox5 and Sox10 in fate specification of pigment cells in medaka and zebrafish. PLoS Genet. *14*, e1007260.
- Nonomura, K., Lukacs, V., Sweet, D.T., Goddard, L.M., Kanie, A., Whitwam, T., Ranade, S.S., Fujimori, T., Kahn, M.L., and Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc. Natl. Acad. Sci. USA *115*, 12817-12822.
- Tominaga, J., Nakahara, Y., Horikawa, D., Tanaka, A., Kondo, M., Kamei, Y., Takami, T., Sakamoto, W., Unno, K., Sakamoto, A., and Shimada, H. (2018). Overexpression of the protein disulfide isomerase AtCYO1 in chloroplasts slows darkinduced senescence in Arabidopsis. Plant Biol. *18*, 80.

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

- Amemiya, S., Hibino, T., Minokawa, T., Naruse, K., Kamei, Y., Uemura, I., Kiyomoto, M., Hisanaga, S., and Kuraishi, R. Development of the coelomic cavities in larvae of the living isocrinid sea lily *Metacrinus rotundus*. Acta Zool. 2018 Sep 23.

● *Research activity by Y. Kamei*

Specially Appointed Associate Professor: *KAMEI, Yasuhiro*
NIBB Research Fellow: *SAKAMOTO, Joe*
CREST Researcher: *KAMIKAWA, Yuko*
Technical Assistant: *NAKAGAWA, Mami*
TAMADA, Tomoko

Our research group promotes two cutting-edge microscope projects: “observation” and “manipulation” using optical and biological technologies.

The aim of our “observation project” is seeing deep into 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 Earth based telescopes may be disturbed by fluctuations in the atmosphere, AO technology can mitigate this disturbance. However, living materials have particular refractive indexes, so some organelles may hinder the ideal optical path for microscope observation, similar to the situation with the atmosphere and 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 the observation of living organisms in collaboration with Dr. Tamada of NIBB and Dr. Hayano of the National Astronomical Observatory of Japan (NAOJ) and subsequently acquired high-resolution bright field and fluorescent images of living cells. Our results indicated that improvements in optical resolution were restricted to a small area which is called the “isoplanatic patch”.

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 as such, 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), that has been 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 a heat shock

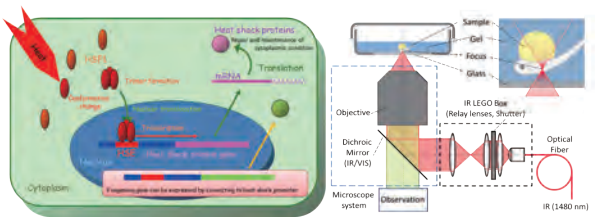


Figure 1. Schematic illustration of heat shock response of cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

response at the desired time. In 2015, we also confirmed the system was effective in the moss *Marchantia polymorpha* and in the newt *Pleurodeles waltl*.

Optimal heating induces a 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 showed that the temperature of the target area rose rapidly and kept a constant level dependent on IR laser power. Furthermore, the heated area was as small as the size of a typical cell.

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, while 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. We then tried this 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 of these species as was 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 a 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 the zebrafish spinal cord (Zeng *et al*. Biol. Cell 2016). In addition, the IR-LEGO system can be utilized for biothermology, a new field that examines temperature or heat in biological systems, because spatiotemporal micrometer order local heating is difficult to perform without this system. We are currently trying to estimate the thermal properties of cells and biomaterials

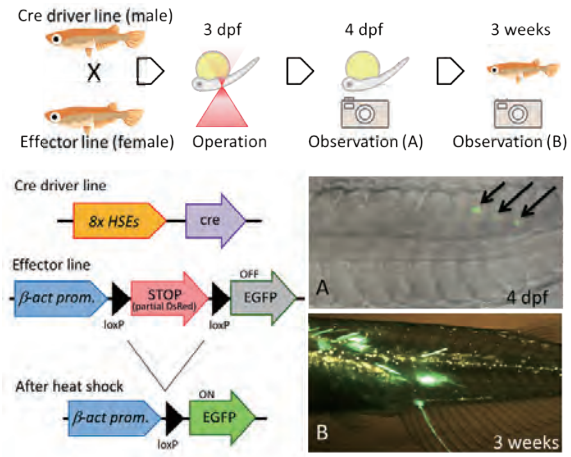


Figure 2. Long-term gene expression system with cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

in vivo using a newly developed thermo-probe (Nakano *et al*. PLoS One 2017) and the IR-laser heating microscope system. We held the 3rd Biothermology Workshop 2018 in Okazaki to build up the new basic biology field with other scientific fields, (such as chemistry and physics) and mathematics in parallel with the above mentioned research.

Publication List:

〔Original papers〕

- Hamada, T., Yako, M., Minegishi, M., Sato, M., Kamei, Y., Yanagawa, Y., Toyooka, K., Watanabe, Y., and Hara-Nishimura, I. (2018). Stress granule formation is induced by a threshold temperature rather than a temperature difference in Arabidopsis. J. Cell Sci. jcs216051.
- Hasugata, R., Hayashi, S., Kawasumi-Kita, A., Sakamoto, J., Kamei, Y., and Yokoyama, H. (2018). Infrared laser-mediated single-cell-level gene induction in the regenerating tail of *Xenopus laevis* tadpoles. Cold Spring Harb. Protoc. *2018(12)*:pdb.prot101014.
- Kosugi, M., Maruo, F., Inoue, T., Kurosawa, N., Kawamata, A., Koike, H., Kamei, Y., Kudoh, S., and Imura, S. (2018). A comparative study of wavelength-dependent photoinhibition of drought-tolerant photosynthetic organisms in Antarctica and the potential risks of photodamage in the habitat. Ann. Bot. *122*, 1263-1278.
- Matsuo, M., Ando, Y., Kamei, Y., and Fukamachi, S. (2018). A semi-automatic and quantitative method to evaluate behavioral photosensitivity in animals based on the optomotor response (OMR). Biol. Open 7, bio033175.
- Nagao, Y., Takada, H., Miyadai, M., Adachi, T., Seki, R., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Hibi, M., Kelsh, R.N., and Hashimoto, H. (2018). Distinct interactions of Sox5 and Sox10 in fate specification of pigment cells in medaka and zebrafish. PLoS Genet. *14*, e1007260.
- Tominaga, J., Nakahara, Y., Horikawa, D., Tanaka, A., Kondo, M., Kamei, Y., Takami, T., Skakamoto, W., Unno, K., Sakamoto, A., and Shimada, H. (2018). Overexpression of the protein disulfide isomerase AtCYO1 in chloroplasts slows darkinduced senescence in Arabidopsis. BMC Plant Biol. *18*, 80.
- Tonoyama, Y., Shinya, M., Toyoda, A., Kitano, T., Oga, A., Nishimaki, T., Katsumura, T., Oota, H., Wan, M.T., Yip, B.W.P., Helen, M.O.L., Chisada, S., Deguchi, T., Au, D.W.T., Naruse, K., Kamei, Y., and Taniguchi, Y. (2018). Abnormal nuclear morphology is independent of longevity in a zmpste24-deficient fish model of Hutchinson-Gilford progeria syndrome (HGPS). Comp. Biochem. Physiol. C Toxicol. Pharmacol. *209*, 54-62.
- Watanabe, Y., Furukawa, E., Tatsukawa, H., Hashimoto, H., Kamei, Y., Taniguchi, Y., and Hitomi, K. (2018). Higher susceptibility to osmolality of the medaka (*Oryzias latipes*) mutants in orthologue genes of mammalian skin transglutaminases. Biosci. Biothechnol. Biochem. *82*, 1165-1168.

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

- Amemiya, S., Hibino, T., Minokawa, T., Naruse, K., Kamei, Y., Uemura, I., Kiyomoto, M., Hisanaga, S., and Kuraishi, R. Development of the coelomic cavities in larvae of the living isocrinid sea lily *Metacrinus rotundus*. Acta Zool. 2018 Sep 23.

DATA INTEGRATION AND ANALYSIS FACILITY

Assistant Professor: *UCHIYAMA, Ikuo*
Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
SUGIURA, Hiroki
Technical Assistant: *OKA, Naomi*

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence, expression data, 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 the development of data analysis pipelines, construction of databases, and website setup to distribute data worldwide as well as providing basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the institute's network systems and provides 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 (HPE Apollo r2800, 20 nodes/800 cores, 192 GB memory/node), a shared memory parallel computer (HPE ProLiant DL560, 72 cores, 3TB memory; HP ProLiant DL980 G7, 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700X, 1.52PB+880TB), 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 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. We have especially provided support in 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 ORION network connecting the three research institutes in Okazaki. Many local services, including sequence analysis, file sharing, 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 conducted by Ikuo Uchiyama

Assistant Professor Ikuo 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.

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 technologies. Such marking allows us to conduct detailed studies of genes and cell functions. Because these model organisms mature in a short period of time, changes in cells, organs, and individuals can be thoroughly and efficiently observed. On this front, the NIBB BioResource Center has the equipment, facilities, and staff to safely, efficiently, and appropriately maintain such organisms.

Model Animal Research Facility

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

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 which are generated using genetic engineering technology, and harness techniques such as 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"; a place where technical and supporting staff develop and promote research-supporting activities. Furthermore, 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 spaces 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 ever since. The new center facility building in the Yamate area has strengthened research activities that require 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 small transgenic fish and birds.

Figure 2. Large sized autoclave in the Yamate area.

In 2018, 3,197 mice (8 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 49,376 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 after microbiological cleaning using *in vitro* fertilization-embryo

transfer techniques (11 transgenic lines), and stored using cryopreservation (23 transgenic lines). The frozen eggs of 1 mice line were taken out of the facility.

Genome editing experiments were performed on two 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 was opened at the beginning of 2005. The facility provides research-supporting activities within 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 2018, 8 mice were brought into the facility in the Myodaiji area, and 826 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 provided 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 1 liter tanks and 450 3 liter tanks are available for medaka and zebrafish, respectively. Water can be maintained to suit the conditions desired for fish breeding. 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 2018, 0 zebrafish (0 fertilized eggs) were brought to the facility. There were no fertilized eggs or chicken embryos brought in or taken from the laboratory for chick embryos 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, Dr. Eiji Watanabe, is the principal investigator of the Laboratory of Neurophysiology. The Laboratory of Neurophysiology is studying various mechanisms of the visual system using a psychophysical approach. For more details, please refer to the page of the laboratory.

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 qualify for housing in other facilities.

The Plant Culture Laboratory equips and manages around 75 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 external and internal 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 which are exposed to precise light concentrations. 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 two flow cytometry systems and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available. In addition, a liquid handling system for fully automated *in situ* hybridization of sections of up to 60 glass slides simultaneously is also provided.

A 386-m^2 experimental farm next to the institute building of the Myodaiji area is maintained for Japanese morning glory and related *Ipomoea* species, several carnivorous plants and other flowering plants that must be cultivated outdoors. Three heated greenhouses (44, 44, and 45 m^2) are used for the sensitive carnivorous plants. Four air conditioned greenhouses (4, 6, 9, and 9 m^2) with air-conditioning are provided for the cultivation of rice *Oryza* sp., *Lotus japonica* and other related legume species, as well as Japanese morning glory mutant lines. Two air conditioned 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 a storage area and workspace. Part of this building is used for rearing of the orchid mantis and the Japanese rhinoceros beetle.

In 2018, several culture boxes were replaced and newer ones were introduced in their place. Of these, a culture box can irradiate extremely bright light (max 76,000 lux) via the use of high luminance LED lamps.

The assistant professors of this facility, Dr. A. Hoshino and Dr. K. Tsugane are the principal investigators of each group in the Laboratory of Biological Diversity. For more details, please refer to the page of the laboratory.

Figure 5. A plant culture box with high luminance LED lamps.

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 6. 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 for life sciences research. The project also aims to improve these bioresources by increasing their value by enriching their genome information and developing key preservation technologies and other necessary procedures, in order to meet current scientific demands. NIBB serves as the core organization center of medaka bioresources and as a sub-center of morning glory and the zebrafish bioresources.

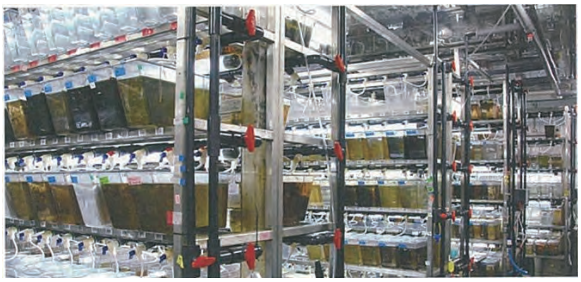
I. NBRP Medaka (*Oryzias latipes*)

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

NBRP Medaka provides three groups of resources 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 enzymes necessary for manipulation and live imaging of the medaka embryos. Entries for these resources can be found by various methods such as keyword searches, sequence homologies, and by opening the expression profile on the following 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. Furthermore, we have developed and provided a new genome browser (http://viewer.shigen.info/medaka/index.php) that uses a recently published genome assembly by PacBioRSII.

As one of our main accomplishments over the last ten years, we sequenced both ends of 260,000 clones originating from 11 kinds of full-length cDNA libraries and then sequenced the whole length of 17,000 independent clones from 2007 and 2009. We also developed strains in which CRE-recombinase can be expressed in any of the cell lineages using a heat shock promoter. We then started to provide strains (TG918, TG921, *etc*) established using this method. By 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 service aimed at preserving testicular tissues using this method.



NBRP Medaka fish facility, which is used to collect, maintain and supply live medaka.

II. NBRP Morning Glory (*Ipomoea nil*)

Project Manager: *HOSHINO, Atsushi*

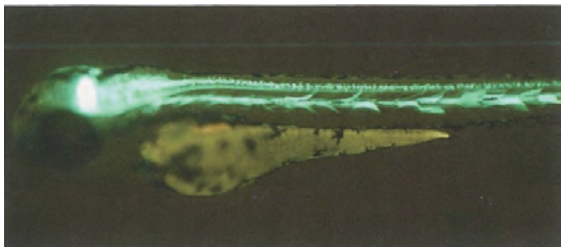
The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, and is studied worldwide, especially in plant physiology and genetics. NIBB collects, develops and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning Glory, and collaborates with the core organization center, Kyushu University. We have collected several mutant lines and DNA clones, and provided 9 mutant lines and 37 DNA clones to both local and international biologists during the course of 2018.

The database (http://shigen.nig.ac.jp/asagao/) contains genotype data and thousands of image data of phenotypes for 1,200 *Ipomoea* lines maintained by this project.

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 mainly collect zebrafish strains expressing fluorescent proteins in specific cells of the central nervous system and distribute them to researchers worldwide. The zebrafish is an important and globally used experimental vertebrate model animal with a simple body structure. It can be genetically manipulated, and its embryos are transparent enough for optical observations. Research 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.

‡: NBRP Zebrafish was moved to the charge of the ExCELLS from 1 April 2018.

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

Head and Specially Appointed Professor:
NARUSE, Kiyoshi

Specially Appointed Assistant Professor:
TAKETSURU, Hiroaki

Technical Assistant:
AKIMOTO-KATO, Ai
TANAKA, Ayako
MATSUBAYASHI, Naomi
MIZOKAMI, Yuko
TSUZUKI, Chizuru

In order to realize the vision of a life science community that can withstand 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 finalized 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 have 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.

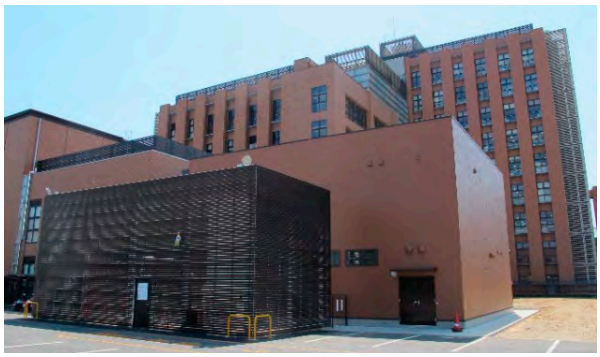


Figure 1. IBBP Center.

The IBBP Center includes: earthquake proof structures capable of withstanding even very large scale quakes which are 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 preservation methods used are the freezing of animal sperm and eggs, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

When university satellite hubs receive preservation requests involving biological resources from researchers, they report to the Managing Project Committee of IBBP (which is comprised of NIBB faculty members and other satellite institutes), where the relevance of the request is reviewed. If the request is approved, the biological resources that are to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated), and their particulars 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 returned 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 IBBP Center's state of the art facilities. As a result of this, Japan's research infrastructure has been significantly strengthened.

I. Current status of back up available for biological resources

In 2018, the IBBP Center stored 5,468 384-well and 112 96-well plates consisting of 2,110,464 clones as cDNA/BAC clones, 15,960 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 4,503 133mm-straw tubes for sperm and 654 seed samples. In total 2,110,464 samples were stored.

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

Figure 3. Cryo tube 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 biological resources for which cryopreservation methods are not well established.In order 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 focuses on two goals: 1) the 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 2018, we have conducted 18 collaborative research projects aimed at achieving these goals. We also worked to establish a research center for cryo-biological study thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2018 on October 25-26, 2018 at the Okazaki Conference Center in Okazaki, Japan. We had 108 participants from several fields covering physics, chemistry, biology, and technology.

Figure 4. Group photo of Cryopreservation conference 2018.

RESEARCH ACTIVITY BY H. TAKETSURU

Specially Appointed Assistant Professor:
TAKETSURU, Hiroaki

Vitrification of rat embryos at various developmental stages

Numerous genetically engineered rat strains have been produced via genome editing. Although the freezing of embryos is helpful for the production and storage of these valuable strains, the tolerance to freezing of embryos varies at each developmental stage of the embryo. This study examined the tolerance to freezing of rat embryos at various developmental stages, particularly at the pronuclear stage. Embryos that had developed to the pronuclear, 2-cell, and morula stages were frozen via vitrification using ethylene glycol- and propylene glycol-based solutions (Figure 1). More than 90% of the embryos at all developmental stages survived after warming. The developmental rates of offspring

Figure 1. Vitrification and transfer of rat embryos.

of thawed embryos at the pronuclear, 2-cell, and morula stages were 19%, 41%, and 52%, respectively. Pronuclear stage embryos between the early and late developmental stages were then vitrified. The developmental rates of offspring of the thawed pronuclear stage embryos collected at 24, 28, and 31h after the induction of ovulation were 17%, 21%, and 23%, respectively. These results indicated that the tolerance to vitrification of rat embryos increased with the development of the embryos. The establishment of a vitrification method for rat embryos at various developmental stages is helpful for improving the production and storage of valuable rat strains used for biomedical science.

Figure 2 shows the developmental ability of embryos at various developmental stages. The developmental rates of offspring of fresh embryos collected at pronuclear, 2-cell, and morula stages were 54%, 53%, and 45%, respectively. More than 90% of embryos survived freezing, and 19%, 41%, and 52% of the surviving embryos developed to normal offspring after their implantation at the pronuclear, 2-cell, and morula stages, respectively. The rate of implantation and development to offspring was significantly lower in the pronuclear stage embryos than in the 2-cell and morula stage embryos after freezing.

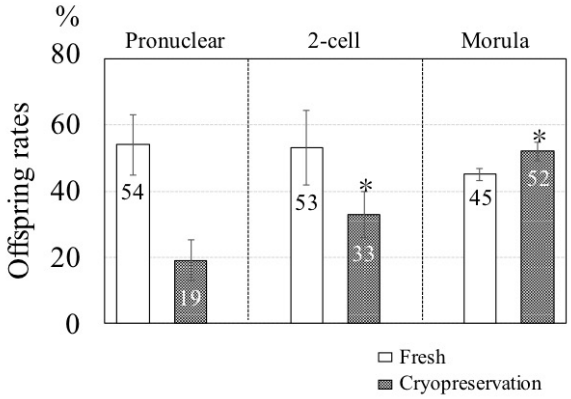


Figure 2. Development of rat embryos at various developmental stages.

The developmental ability of pronuclear stage embryos after various developmental periods are shown below (Figure 3). Although embryos collected at 24h after hCG injection (control) showed high survival rates (93%) after freezing, these embryos showed low rates of development (28%) when compared to 2-cell embryos. The survival of frozen embryos collected at 28 and 31h after hCG injection was high (both 95%), and they showed a significantly higher (45% and 67%) development to the 2-cell stage compared with the control embryos. The development to offspring (21% and 23%) of these embryos was slightly increased, although no significant difference was seen compared with control embryos. The ability of embryos to survive and develop to the 2-cell stage when cultured in vitro for 4

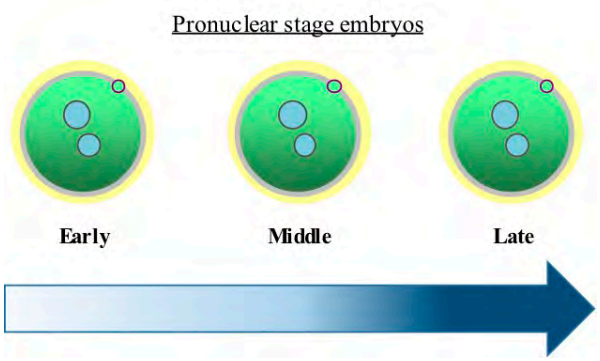


Figure 3. Increase the tolerance to vitrification with embryo development?

or 7h after collection at 24h after hCG injection was also significantly higher than that of control embryos. However, the rate of implantation sites and development to offspring of these embryos were similar to those of embryos collected at 28 and 31h after hCG injection.

Embryo freezing is useful in the production and maintenance of valuable genetically engineered rat strains. Vitrification is helpful because embryos can be rapidly and easily frozen without any specialized equipment, such as a programable freezer. Vitrified pronuclear stage embryos are required for introducing endonucleases during genome editing. Further studies are needed to establish the effects of vitrification on rat embryos at all developmental stages.

Publication List:

〔Original paper〕

● Taketsuru, H., and Kaneko., T. (2018). Tolerance to vitrification of rat embryos at various developmental stages. Cryobiology *84*, 1-3.

CENTER FOR THE DEVELOPMENT OF NEW MODEL ORGANISMS

Head
UENO, Naoto

Specially Appointed Associate Professor
(Cross-appointment with Hiroshima University)
SUZUKI, Ken-ichi

Technical Assistant: *TAKAYAMA, Ayuko*

Since the beginning of life on Earth, living organisms have evolved to adapt to various environments, and have spawned a wide variety of species. Modern biological research has put an emphasis on elucidating the basic principles common to many species, and has progressed thanks to the intensive analysis of species known as model organisms, which are easy to handle in a laboratory environment. However, this development has left many interesting biological phenomena unexamined as their distinctive characteristics are observed only in a particular group of species of model organisms. How we overcome this problem is an important challenge for biology hereafter.

To solve this problem, we need to choose species most suitable to analyze the phenomena of interest, and then establish them as new model organisms by developing new methodologies. These include stable raising, breeding and experimental manipulation techniques, analyses of the genome information and gene expression, and gene manipulation techniques using gene insertion and genome editing techniques.

To this end, The Center for the Development of New Model Organisms was established in 2013 and through its activities; organisms that have been rarely used in research have been recently designated as new model organisms, including aphids and sea anemones, to understand symbiosis phenomena or rhinoceros beetles for studying the evolution of the insects. We are refining various processes ranging from information sharing regarding new model organisms to the development of genetic and phenotype analysis and genetic engineering, and aim to seamlessly fashion these steps into a continuous research flow.

Research activity by K. Suzuki

Specially Appointed Associate Professor
(Cross-appointment with Hiroshima University):
SUZUKI, Ken-ichi

Two technical innovations have recently changed biology: Next Generation Sequencing (NGS) and Genome Editing. NGS reveals whole genome sequences and gene expression profiles from various organisms. Genome Editing accelerates the functional characterization of numerous genes involved in a variety of phenomena of life. Accordingly, we are now basically able to choose any organism which we are interested in, and carry out functional analyses by using these tools.

I. Development of genome editing techniques for various organisms.

Recent advances in the CRISPR-Cas system now allow for reverse genetics in various organisms. However, it has been hampered by the lack of a simple and efficient method for gene modification in most of the non-model organisms. To overcome this problem, we developed a highly-efficient workflow for gene knockout in the founder using this CIRSPR-Cas. We call the virtually knockout founders “crispants”. Crispant assay provides us with a practical and rapid tool for functional screening of numerous genes of interest beyond the post-genome era (Figure 1).

Figure 1. *tyrosinase* crispant in *P. waltl*. A knock-out founder of tyrosinase, a melanin synthesis enzyme, and wild newt (left and right, respectively). *tyr* crispant shows full albinism.

Despite the practical utility of the knockout technique, there is still room for improvement in the integration of exogenous DNA into a target chromosomal site (*i.e.* knock-in), which is still somewhat limited in various organisms. Therefore, we are currently developing more efficient and practical knock-in techniques than conventional ones.

II. Finding new model organisms and deciphering organ regeneration

One of our missions is to discover unique organisms and develop them as new model organisms for basic biology. A recent example of this is our recent establishment of the newt *Pleurodeles waltl* as an experimental model animal for regenerative biology using NGS and Genome Editing techniques. *P. waltl* possesses several excellent characteristics as a model animal: easy breeding, short sexual maturation period, remarkable regenerative capacity and comparatively high efficiency of genome-editing (Figure 2). We are currently investigating the molecular basis of organ regeneration using this newt. In addition, we widely support researchers who attempt to develop new model organisms contributing to up-coming biology.

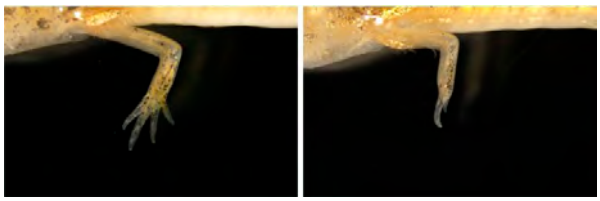


Figure 2. A limb-specific enhancer (ZRS/MFCS1) of *sonic hedgehog* crispant in *P. waltl*. Phenotypes of limb regeneration in wild and ZRS/MFCS1 crispant (left and right, respectively). Unlike in normal limb regeneration in the wild type, severe reduction of digit formation was seen in ZRS/MFCS1 crispant.

Publication List:

〔Original paper〕

- Suzuki, M., Hayashi, T., Inoue, T., Agata, K., Hirayama, M., Suzuki, M., Shigenobu, S., Takeuchi, T., Yamamoto, T., and Suzuki, K. (2018). Cas9 ribonucleoprotein complex allows direct and rapid analysis of coding and noncoding regions of target genes in *Pleurodeles waltl* development and regeneration. Dev. Biol. *443*, 127-136.

〔Review article〕

- Suzuki, K., Sakane, Y., Suzuki, M., and Yamamoto, T. (2018). A simple knock-in system for *Xenopus* via microhomology mediated end joining repair. Methods Mol. Biol. *1865*, 91-103.

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 support 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 waste to that organization.

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

The following are notable activities that CRF conducted in 2018:

1. Renewal of radiation worker registration forms and health check interview sheets

In April 2018, all radiation worker registration forms and health check interview sheets were renewed. The main changes added were the ability to complete the forms in English, and a reduction in the number of forms.

2. Installation of an air-conditioner in the radioisotope storage room in the Yamate area

Before installing the additional air-conditioner, we were running the air-conditioner across the entirety of the radiation controlled area to prevent temperature rises in the radioisotope storage room during summer. Therefore, we installed the air-conditioner in the radioisotope storage room, stopped using the air-conditioner in the radiation controlled area when it is unnecessary, and thus were able to reduce costs by doing so. The dew condensation water generated from the air conditioner is drained to the sink in the radioisotope storage room as it may be contaminated with radioisotopes. (Figure 1A, 1B)

Figure 1. The CRF's notable activities in 2018:
A: the indoor air-conditioner unit
B: the outdoor air-conditioner unit (Displayed on the right in photo B)

The number of registrants and the number of users at our facility from January 2018 to December 2018 are shown in Table 1. Users and visitors counted by the access control system in the controlled areas numbered 1,123 during this period. The numbers for each area are shown in Table 2. The annual changes concerning 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. The total of radioisotopes received has decreased further to a greater degree when compared to last year, and the number of radioisotopes received only amounted to 1 at the Myodaiji-area and 7 at the Yamate area. The figures for training courses on radioisotope handling are provided in Table 4.

	Myodaiji Area	Yamate Area
Registrants	46	34
Users	16	16

Table 1. Numbers of registrants and users at the Myodaiji and Yamate areas in 2018.

	Myodaiji Area	Yamate Area	Total
Users	457	407	864
Visitors	104	155	259
Total	561	562	1,123

Table 2. Users and visitors who entered each controlled area in 2018.

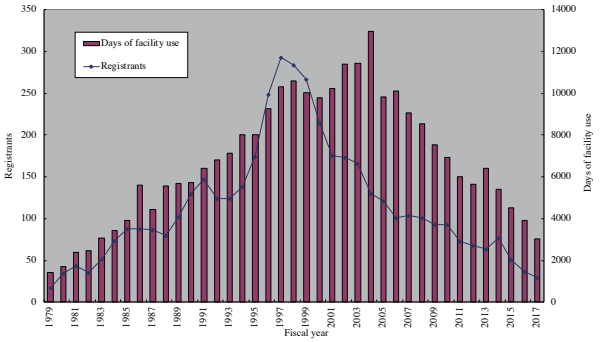


Figure 2. Annual changes of registrants and days of facility use per fiscal year.

		Myodaiji Area	Yamate Area	Total
^{125}I	Received	0	275	275
^{125}I	Used	0	165	165
^{35}S	Received	0	0	0
^{35}S	Used	0	0	0
^{32}P	Received	9,250	9,250	18,500
^{32}P	Used	8,140	0	8,140
^{14}C	Received	0	0	0
^{14}C	Used	0	0	0
^{3}H	Received	0	2,775,000	2,775,000
^{3}H	Used	0	1,850,037	1,850,037

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2018.

training course	place	numbers of participant
Introductory course for beginners	Myodaiji	0
Introductory course for beginners	Yamate	0
Introductory course for experts	Myodaiji	4
Introductory course for experts	Yamate	0
Users training course	Myodaiji	42
Users training course	Yamate	30

Table 4. Training courses for radiation workers in 2018.

RESEARCH ENHANCEMENT STRATEGY OFFICE

Director
UENO, Naoto

Vice-Director:
MANO, Shoji

In 2013, NINS started a research enhancement project 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 research. It would do this by emphasizing the following: 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 and especially young, female or foreign researchers. In line with this strategy, the Research Enhancment Strategy Office was founded to achieve the above mentioned four goals at NIBB.

The Research Enhancement Strategy Office, which consists of six groups with distinct missions, is aimed at supporting researchers so that NIBB can improve its ability as a collaborative research institution and to provide comprehensive support within the organization. It was created in 2013 via the a restructuring of 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) in accordance with recommendations given by a group adviser chosen from the ranks of NIBB's professors and in close collaboration with the NINS's Research Enhancement Promotion Headquarters.

EVALUATION AND INFORMATION GROUP

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

This group is tasked with assisting the Director General in the preparation of 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

Every year, NIBB asks non-NIBB members of the Advisory Committee for Programming and Management to submit a questionnaire concerning research achievements, collaborative research, as well as other activities conducted at the institute. Furthermore, several of these members, and a few of other leading scientists in basic biology, are then invited to participate in an evaluation session pertaining to these activities and to propose future directions for the institute. The record of this meeting is published annually.

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

This group edits the annual report (this publication) which summarizes the annual activities of all of the institute's laboratories and facilities. The annual report is also used to publicize the institute and also for evaluation purposes.

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

This group assists in the preparation of NIBB's Medium-term Goals and Plans which in themselves span a period of six years. We are also tasked with formulating Annual Plans in order to set yearly benchmarks in regards to the progress of the above mentioned Goals and Plans. The department also assists in preparing Business and Performance Reports for external evaluation on whether we are meeting the goals set both annually and over 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 2018

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
COWAN, Glen
Group Advisor: *UENO, Naoto*

One of NIBB's missions is to continually explore the leading-edge of biology and form research communities that link Japan to the world. To achieve this, it holds scientific meetings such as the "NIBB Conferences" and "Okazaki Biology Conferences (OBC)", as well as educational programs such as "NIBB International Practical Courses". Furthermore, NIBB is coordinating closely with the European Molecular Biology Laboratory (EMBL, European member states), the Temasek Life Sciences Laboratory (TLL, Singapore) and Princeton University (USA). Working on the basis of cooperative agreements signed between NIBB and the above listed organizations, NIBB coordinates the exchange of personnel and techniques and the joint staging of scientific meetings. NIBB is also conducting the "NIBB International Collaborative Research Initiative" to promote high-level international collaborations between faculty members and researchers around the world. It also invites leading-edge researchers from abroad to promote academic exchange with NIBB members in addition to starting new international collaborations.

This group supports and coordinates NIBB's activities related to international research collaborations, by organizing the various above-mentioned international scientific meetings and technical courses, coordinating the dispatch of researchers to international conferences, and supporting researchers visiting from the institutes mentioned above. This group also supports NIBB internship students visiting from foreign countries, and the dispatch of SOKENDAI graduate students (the Graduate University for Advanced Studies) to international conferences 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 2018

1) Coordination of international conferences and the International Practical Course

This group coordinated and supported the following international events organized by NIBB:

- The 10th NIBB International Practical Course "Genome Editing and Imaging of Fish and Amphibians" Okazaki, Japan, September 20-29, 2018 (p. 101)
- GBI-ABiS International Training Course for Bioimage Analysis (held in OIST, Okinawa, Japan) October 31-November 4, 2018 (p. 106)

2) Support of visiting researchers to NIBB

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

- One researcher coming from Princeton University (June)
- 12 members coming from the Science and Technology Department of Hubei Province, China (August)
- Six researchers coming from Academia Sinica ICOB, Taiwan (September)

3) Support of education-related programs

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

- NIBB Internship Program 2018 (p. 107)

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 within this Specially appointed associate professors within this group belong to the NIBB core research facilities, and are responsible for managing collaborative research projects and practical courses that take advantage of their expertise in their particular field. It is through this that we are able to further promote the exchange of information and greater collaboration among scientific communities, as well as support the development of new equipment and methods.

In 2018, this group hosted a total of 102 collaborative projects. The success of these collaborations is best illustrated by the 35 research papers that they jointly published during this year. One of the more remarkable cases was the firefly genome project which was conducted across a 5-year timeframe by research teams lead by Drs. Yuichi Oba (Chubu Univ.) and Shuji Shigenobu (NIBB). By sequencing the genomes of the Japanese firefly Heike-botaru, *Aquatica lateralis*, and the North American firefly, *Photinus pyralis*, they revealed the evolutionary process of bioluminescence in these insects (Fallon *et al*., 2018). They discovered that luciferase, the key enzyme behind the glow generated by the above listed fireflies, arose from duplications and divergences of CoA ligase genes involved in fat metabolism, (which in itself is unrelated to luminescence).

● Fallon, T.R., Lower, S.E., Chang, C.-H., Bessho-Uehara, M., Martin, G.J., Bewick, A.J., Behringer, M., Debat, H.J., Wong, I., Day, J.C., Suvorov, A., Silva, C.J., Stanger-Hall, K.F., Hall, D.W., Schmitz, R.J., Nelson, D.R., Lewis, S., Shigenobu, S., Bybee, S.M., Larracuente, A.M., Oba, Y. and Weng, J.-K. (2018). Firefly genomes illuminate parallel origins of bioluminescence in beetles. Elife *7*, e36495.

YOUNG RESEARCHER SUPPORT GROUP

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

As a leading international institute in the field of basic biology, one of NIBB's more important missions is the cultivation of future researchers. As such, NIBB provides PhD courses for graduate students while acting as the Department of Basic Biology in the School of Life Sciences at SOKENDAI (the Graduate University for Advanced Studies). To compliment this, The Young Researcher Support Group is aimed at supporting young researchers at NIBB (including graduate students such as SOKENDAI and special research students from other universities) to help make their study and research experiences at NIBB beneficial and valuable for their career.

The main activities of the group

1) Coordination and management of courses provided by the Department of Basic Biology (lectures, research presentations, etc.), in collaboration with all faculty members in NIBB.
2) Support in organizing NIBB's programs related to PhD courses, such as the Open Campus Day for prospective students.
3) Cooperation with the interdepartmental programs offered by the SOKENDAI headquarters, including the Freshman Course and the Life Science Retreat.
4) Gathering and providing information that is useful to 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 careers while enduring the stresses of life events, such as having children, this group manages the research assistant system for those in need, and promotes networking among female researchers.

In 2018, a multi-purpose room within the NIBB building was refurbished to suit the needs of NIBB members who may occasionally want to bring their children to work. One of the major refurbishments was to create a "shoes-off space" so that young children can play safely on the floor. Upon the trial period commencing in May, the room is now available for all registered NIBB members. As of writing, 17 members have been registered. The room has also been particularly useful for those wishing to hold meetings or to wait for one's partner/spouse while s/he is in the lab in cases where alternative child care service cannot be arranged.

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

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

NIBB-Princeton University Collaboration Associate Professor Rebecca D. Burdine's visit to NIBB

Associate Professor Rebecca D. Burdine from the Department of Molecular Biology at Princeton University, visited NIBB on June 11th 2018 to conduct a seminar and to meet with NIBB researchers.

Prof. Burdine has been conducting an international joint research project in collaboration with the Division of Morphogenesis at NIBB, and which has been financially supported by NINS. In this capacity, she conducted an exclusive seminar titled "New Roles for Motile Cilia in Development and Disease" for NIBB researchers during her stay in Okazaki. She also had a meeting with the members of the Division of Morphogenesis to discuss the future of the abovementioned joint research project.

In addition to this, Prof. Burdine discussed research with other NIBB researchers and toured the Laboratory of Spatiotemporal Regulation to observe a light-sheet laser scanned microscope.

The 10th NIBB International Practical Course "Genome Editing and Imaging of Fish and Amphibians"

September 20 (Thu) – 29 (Sat), 2018

- Participants: 9 (1 from the UK, Colombia, Nepal, Korea, China, Taiwan respectively, and 3 Japan)
- Venue: National Institute for Basic Biology, Okazaki, Japan
- Seminar Speakers:
  Dr. Lazaro Centanin (Heidelberg Univ., Germany)
  Dr. Shoji Fukamachi (Japan Women's Univ.)
  Dr. Pung-Pung Hwang (Academia Sinica, Taiwan)
  Dr. Takeshi Igawa (Hiroshima Univ.)
  Dr. Asano Ishikawa (National Institute of Genetics)
  Dr. Shinya Komoto (OIST)
  Dr. Haruki Ochi (Yamagata Univ.)
  Dr. Takuji Sugiura (IMP, Austria)
  Dr. Hitoshi Yokoyama (Hirosaki Univ.)
- Lecturers:
  Dr. Yoriko Ando (Nagoya Univ.)
  Dr. Satoshi Ansai (NIBB)
  Dr. Shoji Fukamachi (Japan Women's Univ.)
  Dr. Toshinori Hayashi (Tottori Univ./iNewt)
  Dr. Aiko Kawasumi (RIKEN BDR)
  Dr. Yasuhiro Kamei (NIBB/ABiS)
  Dr. Shigenori Nonaka (NIBB/ABiS)
  Dr. Kiyoshi Naruse (NIBB/NBRP-Medaka/IBBP)
  Dr. Hajime Ogino (Hiroshima Univ./NBRP-*Xenopus*)
  Dr. Ken-ichi T. Suzuki (Hiroshima Univ./NIBB/iNewt)
- Course Staff:
  Dr. Ying-Jey Guh (Nagoya Univ.), Ms. Maki Kondo (NIBB), Ms. Megumi Matsuo (Japan Women's Univ.), Mr. Yu Murakami (Kyoto Univ.), Ms. Misako Saida (NIBB), Dr. Joe Sakamaoto (NIBB), Ms. Nanoka Suzuki (Hiroshima Univ.), Dr. Atsushi Taniguchi (NIBB/ABiS), Dr. Yuka Taniguchi-Sugiura (IMP, Austria)
- Contents of the course:
  Husbandry (Lectures and Experiments Using Medaka, *Xenopus* and Iberian Ribbed Newt)
  Genome Editing (Lectures and Experiments Using Medaka, *Xenopus* and Iberian Ribbed Newt)
  Molecular Works for Genome Editing (Lectures)
  Microinjection (Lectures and Experiments Using Medaka, *Xenopus* and Iberian Ribbed Newt)
  Regeneration Experiments (Lectures and Demonstrations Using Amphibians)
  Cryopreservation/Artificial Insemination (Lectures and Experiments Using Medaka and *Xenopus*)
  Imaging: Light-Sheet Microscope/Laser Confocal Microscope (Lectures and Experiments Using Medaka and *Xenopus*)
  In vivo Cell Manipulation (IR-LEGO) (Lectures and Experiments Using Medaka and *Xenopus*)
  Behavioral Assay (Lectures and Experiments Using Medaka)

The 10th NIBB International Practical Course was held from 20th to 29th September, 2018. The title of the course was “Genome Editing and Imaging of Fish and Amphibians”, and as such medaka, *Xenopus* and Iberian ribbed newt were used during this course. The course itself contained segments concerning “Gene knock-out and knock-in by the CRISPR-Cas9 method”, “Imaging analysis using a light-sheet microscope and a confocal microscope”, “Gene expression induction using the IR-LEGO system”, “Cryopreservation of medaka sperm” and “Optomotor response analysis of medaka using large spectrograph and data analysis of the response” among others. 10 open seminars by invited speakers from Japan and abroad were also held. Dr. Pung-Pung Hwang (Institute of Cellular and Organismic Biology (ICOS), Academia Sinica, Taiwan), Dr. Lazaro Centanin (Centre for Organismal Studies, University of Heidelberg, Germany) and Dr. Takuji Sugiura (Research Institute of Molecular Pathology, Austria) were invited to lecture, and they conducted both seminars and course lectures.

The 11th NIBB International Practical Course co-organized by NIBB and Academia Sinica will be held in Taipei, Taiwan in 2020. To discuss several matters related to the next course, six researchers including Dr. Hwang visited NIBB from ICOS during the course. They visited several laboratories and facilities during their visit and presented their research during the NIBB Special Seminar.

Moving into the future, we hope that the NIBB International Practical Course will be an opportunity to start as well as advance international collaborations and cooperation at various levels.

On behalf of the organizers
Kiyoshi Naruse (NIBB)
Hajime Ogino (Amphibian Research Center, Hiroshima University)

The 12th NIBB Bioimaging Forum "The Future of Bioimage Analysis Explored by AI"

Organizing Committee: Yasuhiro Kamei, Yoshitaka Kimori, Kagayaki Kato, and Natsumaro Kutsuna

The 12th NIBB Bioimaging Forum was held on March 26th 2018. We have previously held a series of forums on advanced bioimaging technologies such as novel techniques of image analysis, the application of adaptive optics to light microscopy, the imaging of physical properties *etc*. However, this time we picked artificial intelligence (AI), an area that has been growing rapidly in recent years, as the main subject of the forum.

We organized the forum so that the participants could understand how AI will affect the field of bioimaging in the near future and feel "the future of bioimage analysis explored by AI". The importance of imaging in biology is continually increasing, but at the same time we have come to feel the presence of its various limitations. For example, as the spatial and the temporal resolution of microscopic images has increased, the size of the data has vastly expanded to the point where we require computer science to analyze it. As AI will most likely play a key role in future data analysis, there must be a mutual understanding and collaboration between biology and computer science so we can be prepared for the future.

While this forum included an introduction to the cutting edge of AI technologies and lectures conducted by image analysis professionals, it also featured lectures held by biologists on how they are trying to apply AI to the analyses of biological phenomena. At the end of the forum we had general discussions and provided an opportunity for mutual understanding between image analysis professionals and lecturers from IT industries.

From last year, this forum has been supported by the Grant-in-Aid for Scientific Research on Innovative Areas - Platforms for Advanced Technologies and Research Resources "Advanced Bioimaging Support (ABiS)". We would like to thank members of the ABiS image analysis support team for their lectures.

(Yasuhiro Kamei)

The NIBB Genome Informatics Training Course

The NIBB Core Research Facilities regularly 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 2018, we held two sets of training courses on RNA-seq analysis and a course on BLAST analysis. The entirety of the RNA-seq analysis course was made up of two 2-day programs: one was a preparatory course concerning the basics of UNIX and R, and the other was a practical course to learn the pipelines of RNA-seq analysis using next-generation sequence data. The BLAST analysis course was designed for advanced users and aimed at enabling participants to become more familiar with large scale sequence database searches in a 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.), Dr. Katsushi Yamaguchi, Ms. Hiroyo Nishide, Mr. Takanori Nakamura, Mr. Takahiro Bino (NIBB Core Research Facilities)

February 22(Thu)-23(Fri), 2018

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

- 22 participants (including 1 from NIBB)
- Program:
  1. UNIX for Beginners
  2. Editor and Scripts
  3. Introduction to "R"
  4. NGS Basic Data Formats and NGS Basic Tools
  5. Text Processing
  6. Exercises

March 8(Thu)-9(Fri), 2018

(Practical Course) RNA-seq Analysis Pipeline
(Same participants as above)

- Program:
  1. Introduction to RNA-seq
  2. NGS Basic Data Format and Basic Tools
  3. Visualization of NGS Data
  4. Introduction to Statistics
  5. RNA-seq Pipelines: Genome-Based and Transcriptome-Based Approaches
  6. Multivariate Statistics
  7. Functional Annotation and Gene Ontology
  8. Exercises

July 5(Thu)-6(Fri), 2018

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

- Participants: 22 participants (including 1 from NIBB)

July 26(Thu)-27(Fri), 2018

(Practical Course) RNA-seq Analysis Pipeline
(Same participants as above)

Mastering BLAST: The Essence of Sequence Analyses

September 6(Thu)-7(Fri), 2018

- Organizers & Lecturers: Dr. Shuji Shigenobu, Dr. Ikuo Uchiyama (NIBB Core Research Facilities)
- Participants: 21 participants (including 1 from NIBB)
- Program:

1. BLAST Basics
2. Local BLAST Search with Command-line
3. BLAST Inside: Theoretical Background of Sequence Search
4. Large Scale BLAST Search
5. Sequence Database Functions
6. Annotation of Genes and Ortholog Analysis
7. Beyond BLAST
8. Exercises

The 5th BioImaging Analysis Training Course

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

December 4 (Tue) -6 (Thu), 2018

The 5th Bioimaging Training Course was held jointly by the Exploratory Research Center on Life and Living Systems (ExCELLS), JSPS KAKENHI Platforms for Advanced Bioimaging Support (ABiS) and NIBB in December last year. This course was designed for biologists who are relatively new to analyzing datum obtained through advanced microscopy. Therefore, the focus of the training related to learning about 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”. 40 people applied for the course, which had a maximum capacity of 17 participants. This clearly indicated the high demand for courses on these subjects.

This course’s lectures were conducted with the aim of guiding participants towards keeping in mind the series of steps essential to fundamental image processing and analysis while obtaining images to be used (workflows). In addition, we loaned PCs pre-installed with ImageJ, a typical open-source software package for biological image processing and analysis, to the participants in addition to images which were used for practice. Also, lectures were given on how programming of simple “macro language”, which uses these workflows in ImageJ allows automation; itself being a necessity 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, but satisfied”. It certainly is beneficial in terms of their image analysis as they became 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)

The demand for bioimaging has increased in recent years in the field of life science. However, due to advances in imaging technologies such as the diversification and specialization of imaging equipment and their increasingly complex operations, increased equipment and operating 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 newly established framework of the Grant-in-Aid for Scientific Research on Innovative Areas (Group Leader: Prof. Masanobu KANO, NIPS/The University of Tokyo). This program aims to contribute to the further development of academic research in Japan through the respective provision and dissemination of cutting-edge equipment and methodologies to individual KAKENHI (Grants-in-Aid for Scientific Research) research approved by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) under the Grant-in-Aid for Scientific Research on Innovative Areas framework (FY2016-FY2021).

ABiS provides cutting-edge instruments for light microscopy, electron microscopy, magnetic resonance imaging, and other types of methods in cooperation with domestic partner organizations that own and operate multiple types of advanced specialized imaging equipment to provide comprehensive support for advanced imaging in the field of life science. NIBB, together with NIPS, contributes to these endeavors as one of the main headquarters of the ABiS network and is in charge of the following ABiS support activities:

- Light microscopy
  - 4D microscopy administered by Prof. Toshihiko FUJIMOIRI
  - IR-LEGO microcopy administered by Assoc. Prof. Yasuhiro KAMEI
  - DSLM administered by Assoc. Prof. Shigenori NONAKA
- Imaging analysis
  - Development of image processing/analysis algorithms for biological data administered by Prof. Naoto UENO and Assist. Prof. Kagayaki KATO
- Training
  - Training for image analysis administered by Assist. Prof. Yoshitaka KIMORI, Assist. Prof. Hiroshi KOYAMA and Prof. Naoto UENO

To organize and coordinate ABiS activities, two secretariat offices were established at NIBB and NIPS (the former is run by Assoc. Prof. Shoji MANO), respectively, under the control of the general support group (personnel in charge of functions at NIBB include Director-general Prof. Masayuki YAMAMOTO, Prof. Naoto UENO, Prof. Shinji TAKADA and Assoc. Prof. Shoji MANO). General support includes budget planning and management of ABiS activities. In particular, we promote ABiS activities via the official ABiS website and other media so that KAKENHI researchers are able to make full use of the ABiS platform to accelerate their research projects. In addition, we organize technology training sessions, workshops, and symposia to disseminate and share information about advanced imaging technologies. 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.

ABiS joins the Global Bioimaging (GBI) Project

ABiS, a supporting platform for bioimaging in life science research in Japan, is a joint effort of imaging experts affiliated with 20 universities and research institutions nationwide which operate various types of advanced and specialized imaging equipment and aims to provide assistance in the field of life science-related advanced imaging in Japan. NIBB and NIPS are supervising this platform as the core organizations of ABiS.

Euro-BioImaging (EuBI) is the largest and most well-established imaging network in Europe, and has been expanding globally to form the Global BioImaging (GBI) network which boasts participants from other areas such as India, Australia, Singapore, the Republic of South Africa, Canada, Mexico and the USA.

In September 2018, ABiS joined the Global Bioimaging (GBI) project as a representative of the Japanese bioimaging community. A signing ceremony was held at the 3rd Exchange of Experiences (EoE) meeting convened on September 15th, 2018 in Sydney, Australia (Figure 1).

It is hoped that through GBI, ABiS will be able to raise the present quality of support to that of the currently accepted international benchmarks, and that further observations,

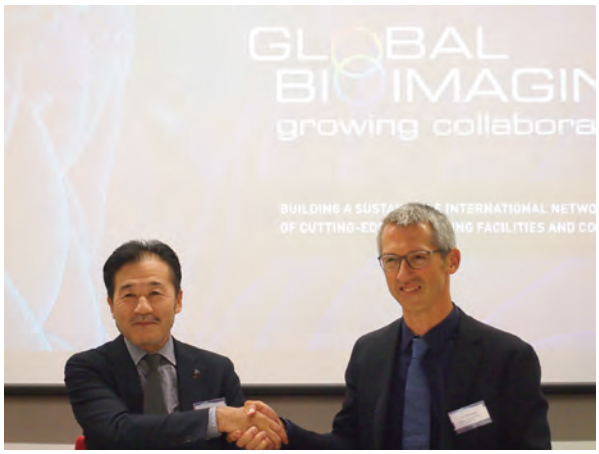


Figure 1. A photo of GBI representative, Dr. Jan Ellenberg and NIBB's Professor Naoto Ueno. Professor Ueno was charged with bringing the agreement signed by NIBB Director General Masayuki Yamamoto and NIPS Director General Keiji Imoto to the EoE.

analysis and research methods will be able to be better shared globally. Furthermore, it is also hoped that this collaboration will lead to a better environment in the bioimaging research field in Japan being set up; one that not only provides training courses for young researchers and the staff of imaging facilities, but also through discussions about the implementation of career paths. GBI also aims to establish a data sharing system infrastructure, promote academic/industrial cooperation and formulate a global strategy for bioimaging research.

Additionally, ABiS and GBI held a symposium, "Frontiers in Bioimaging" and an international training course from October 31 to November 4 in Okinawa as their first joint project together (see below). A subsequent GBI event, "The fifth EoE", will be held in Okazaki, Japan in autumn of 2020.

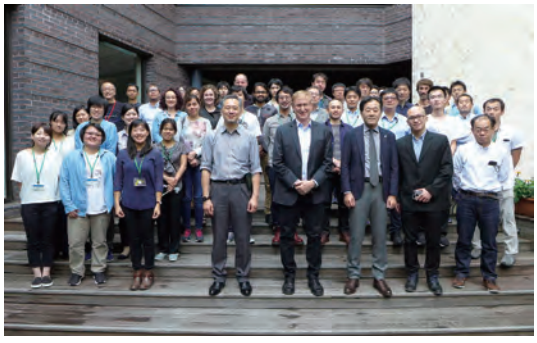
ABiS-GBI-OIST-ResonanceBio Joint Symposium "Frontiers in Bioimaging" and GBI-ABiS International Training Course

October 31 (Wed) ~
November 4 (Sun) 2018

Sponsored by: Advanced Bioimaging Support and Global Bioimaging

- Co-sponsored by:
  Euro-BioImaging
  Okinawa Institute of Science and Technology Graduate Unviersity
  Grant-in-Aid for Scientific Research on Innovative Areas-'Resonance Bio'
  Grant-in-Aid for Scientific Research on Innovative Areas — Platforms for Advanced Technologies and Research Resources "Committee on Promoting Collaboration in Life Sciences"
- Total number of participants: 80
- Oral presentations: 9
- Training course participants: 16

The ABiS-GBI-OIST-ResonanceBio Joint Symposium "Frontiers in Bioimaging" was held in Okinawa to assist in the promotion of information sharing about the forefront of bioimaging. In this symposium, two ABiS members introduced recent research activities related to image analysis which they have been involved in, and two researchers from Global BioImaging (GBI), promoted by Euro-Bioimaging, introduced similar activities in which they have been participants. In addition, three researchers from Grant-in-Aid for Scientific Research on Innovative Areas-'Resonance Bio', and two OIST members gave presentations related to cutting-edge technologies in bioimaging.



Group photo that was taken during the joint symposium.

For four days after the joint symposium, the GBI-ABiS international training course was held to familiarize 16 young researchers/technicians from a variety of nationalities with computational image analysis methodologies by GBI experts.

Group photo that was taken during the international training course.

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 2018 there were 36 applicants, out of which seven interns were selected. These interns were from universities located in six countries (China, Indonesia, Philippines, Thailand, Vietnam, and Japan) and spent periods ranging from two to twelve weeks experiencing life as a member of a research team. Moreover, one intern from the Republic of Serbia stayed at NIBB on two weeks by their own travel grants.

Report from a participant

Sheena Josol
University of Santo Tomas, Philippines

My name is Sheena Josol and I am a Biochemistry graduate from University of Santo Tomas, Philippines. As someone who had just earned her Bachelors degree, I admit to having limited knowledge which I believed is meant to be enhanced. Thus, it is incredibly fulfilling to be selected on this Research internship program hosted by one of the prestigious research facilities in Japan. I was specifically granted a chance to spend my internship in the Division of Quantitative Biology under the supervision of Professor Aoki.

In my 2 months stay, I was given two research projects which independently aimed to (1) reconstitute tumorigenesis and oncogene addiction in a manner dependent to Trimethoprim and (2) dissect molecular mechanisms of stochastic ERK activation, both of which involved the use of wildtype MCF10A cell lines. In the first project, the oncogene of interest that we intended to induce are the ones involved in ERK signaling pathway--the G protein KRas and the protein kinase BRaf specifically its KRasG12V and BRafV600E mutant version respectively which are widely implicated in several human cancers.

The first step in inducing oncogene addiction was to establish the needed cell lines which in this case is a Dihydrofolate reductase (DHFR)-expressing MCF10A cell line. As we all know the best way to ascertain the function of one biomolecule is to remove it from the system and see what happens next. The TMP-DHFR system makes it all possible. This model system works in such a way that it requires the protein of interest to be fused to a destabilizing domain like DHFR which targets it for degradation. The protein is then rescued by the addition of TMP that binds the DHFR and inactivates it which in turn enables it to regulate the stability of protein in a rapid, reversible and tunable manner.

Through imaging, western blotting and cell proliferation assay which I spontaneously did during the course of my internship, I was able to analyze the stabilizing effect induced by TMP , quantify the expression levels of induced oncogenes and examine whether our selected cell line acquire cancer like phenotypes after oncogene induction respectively. As for my second project, due to limited time I was not able to achieve the main goal of studying the stochastic ERK activations.

I am beyond grateful to every member of the lab who humbly imparted their knowledge to me especially to Aoki-sensei and to my mentor Reina-san who patiently taught and guided me with everything. I would also like to express my gratitude to Onoda-san, the secretary in our lab, whose unfailing efforts made my first ever Autumn experience the best. I really appreciate the good laugh I've shared with everyone during lunch time and all the parties they have prepared just for me.

Overall, this experience has not only filled me with significant learnings in the field of science but also let me gain great memories, learn meaningful life wisdom, establish lasting friendship with people from different parts of the world and lastly brought me to wonderful places that made me appreciate the beauty of Japan more. Indeed, Japan is a beautiful country but more than everything else, I think the culture of hardworking, disciplined and kind people makes it more special.

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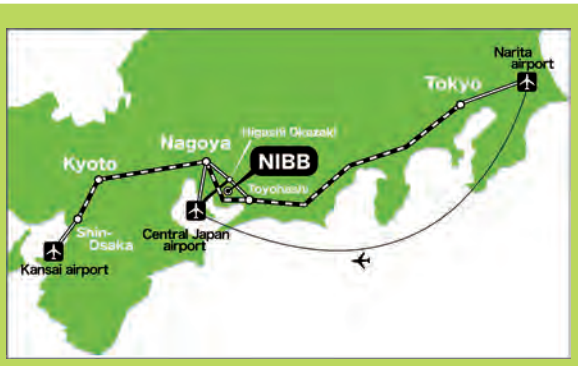
Access



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

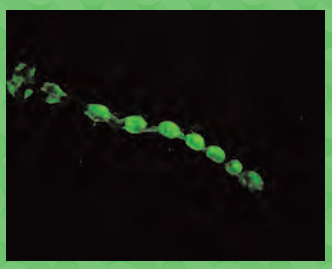
Take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes from the airport).

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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Issued in June 2019
Edited by the Research Enhancement Strategy Office, NIBB