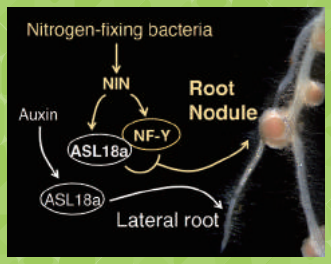

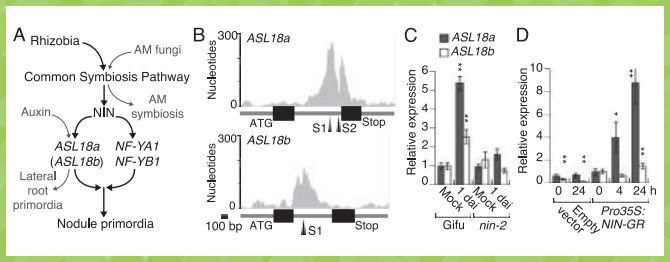

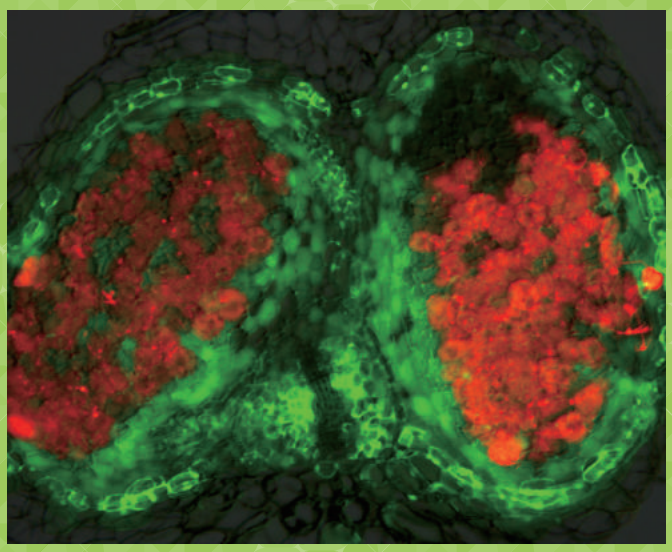

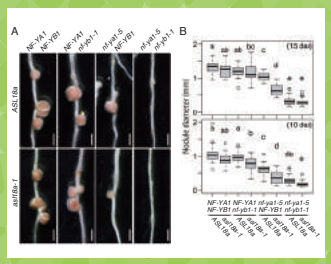

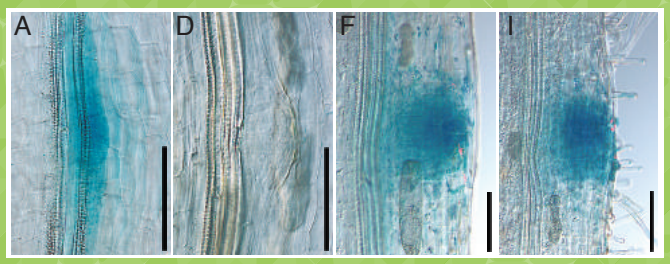

National Institute for Basic Biology

2019 ANNUAL REPORT

Jan. 2019 - Mar. 2020

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Access to NIBB ..... Inside back cover

The cover items are related to a paper titled "A shared gene drives lateral root development and root nodule symbiosis pathways in *Lotus*" (Soyano *et al*., Science 2019) from the laboratory of Prof. Kawaguchi. The paper demonstrated that legumes co-opted a transcription factor involved in early lateral root development for producing root nodules that house symbiotic nitrogen-fixing bacteria. See page 48 of this report for details.

INTRODUCTION

It is my great pleasure to present to you all the National Institute for Basic Biology (NIBB)'s 2019 Annual Report which outlines NIBB's research, educational, and international activities and its effective function as a center for collaborative research in Japan from January 2019 to March 2020.

NIBB aims to establish an international base for biological sciences by using not only model organisms, but also exotic non-model organisms. Consequently, NIBB established the Center for the Development of New Model Organisms in 2014. We have endeavored to develop a one-step pipeline to connect genome sequencing, assembling, and annotation to genome editing using non-model organisms such as carnivorous plants, unique insects, and regenerative newts, and this vision was expounded upon in the April 23rd, 2019 edition of Nature News.

NIBB also developed front bioimaging technologies to acquire high-resolution 3D images and promote quantitative biology in collaboration with Princeton University. In keeping with this, the 66th NIBB Conference/ABiS International Symposium "Cutting Edge Techniques of Bioimaging" and the 2nd NIBB-Princeton Symposium "Imaging and Quantitative Biology" were held in Okazaki. Collaborative studies by researchers from within and external to NIBB have used our bioimaging technologies to generate a lot of fascinating papers (see below).

Concerning changes on the staffing front, Professor Masaharu Noda, an active researcher in the field of integrative neurosciences at NIBB since September 1991, retired in March 2019; roughly one more prior to my appointment as the Director-General of NIBB in April 2019. Associate Professor Shuji Shigenobu was also promoted to the role of taking majority responsibility for managing the Center for the Development of New Model Organisms. Additionally, three assistant professors, two specially appointed assistant professors, and four research fellows were newly posted to NIBB within this year.

Moreover, both I and Vice-Director, Professor Naoto Ueno, visited Heidelberg, Germany in early July and finalized an academic exchange agreement with the Centre for Organismal Studies (COS) in the aforementioned location, and extended the academic exchange agreement with European Molecular Biological Laboratory (EMBL). Following this, "The 67th NIBB Conference/The 6th International "Quest for Orthologs" Meeting was held in September in Okazaki.

I would like to finish this introduction by congratulating Professor Mitsuyasu Hasebe who was awarded the Golden Spore Award 2019 from the International Molecular Moss Science Society.

To establish NIBB as an internationally 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.

Kiyokazu Agata
Director General of NIBB
December, 2020

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.

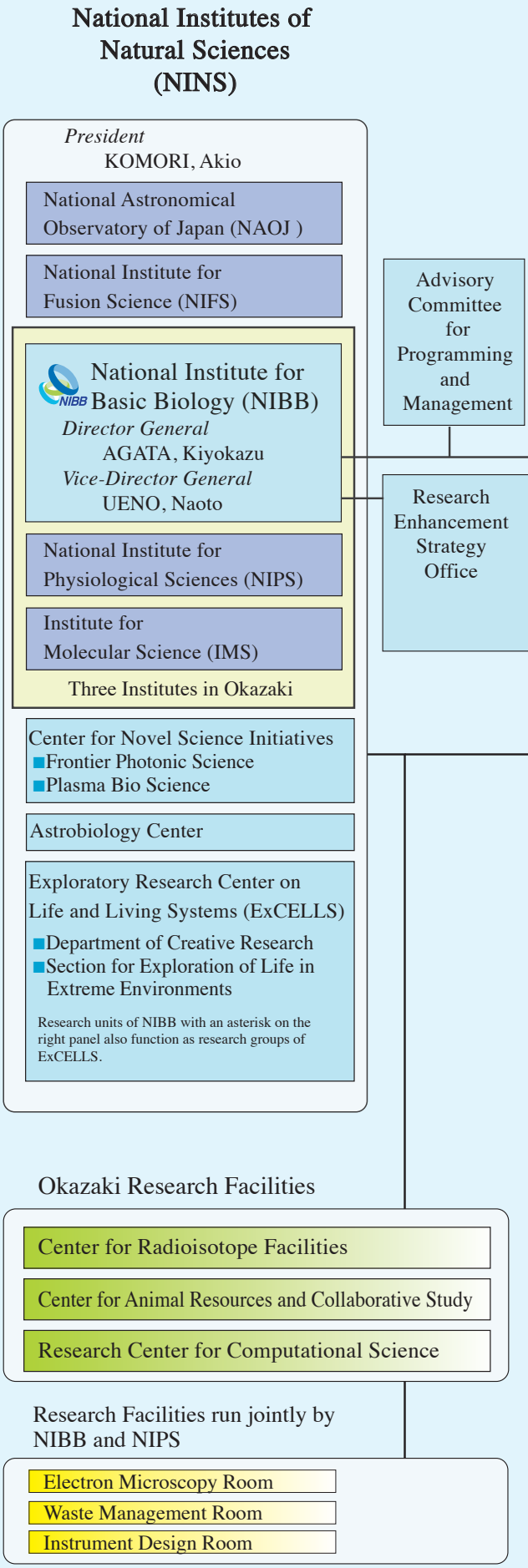
The Research Enhancement Strategy Office, aimed at supporting researchers in order to improve NIBB's abilities as a collaborative research institution, was founded in 2013. The Office is made up of seven groups (p. 96) and its activities are mainly carried out by URAs (University Research Administrators) 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 March 31, 2020

Research Units

Cell Biology	■Division of Cellular Dynamics ■Division of Quantitative Biology* ■Division of Chromatin Regulation ■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 ■Laboratory of Regeneration Biology
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 Evolutionary Genomics ■Laboratory of Bioresources ■Laboratory of Morphodiversity ■Laboratory of Biological Diversity
Environmental Biology	■Division of Environmental Photobiology ■Division of Plant Environmental Responses
Theoretical Biology	■Laboratory of Genome Informatics
Imaging Science	■Laboratory for Spatiotemporal Regulations* ■Laboratory for Biothermology

Research Support Facilities

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

Technical Division

Section of Health and Safety Management

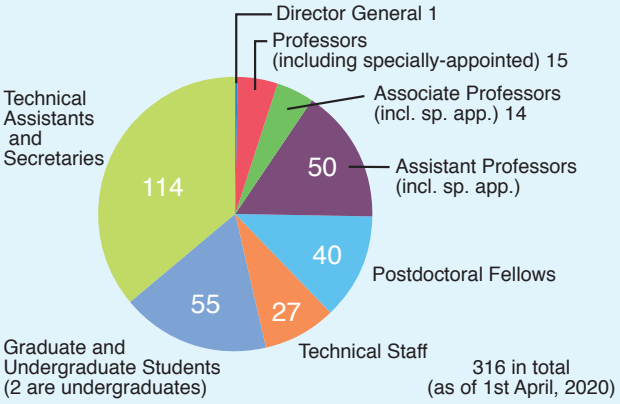
■Evaluation and Information Group
■Public Relations Group
■International Cooperation Group
■Collaborative Research Group
■Young Researcher Support Group
■Gender Equality Promotion Group

Okazaki Administration Center

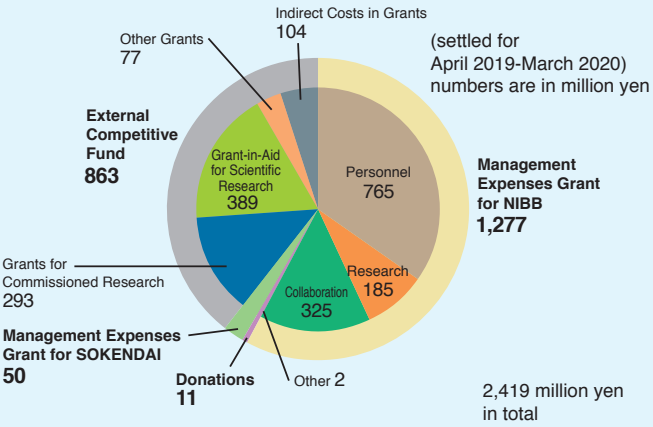
Research Facilities and NIBB BioResource Center were launched in 2010 to support research in NIBB and also to act as an intellectual hub to promote collaboration among the researchers of NIBB and other institutions. The NIBB Center of the Inter-University Bio-Backup Project (IBBP Center) was founded 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 are 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



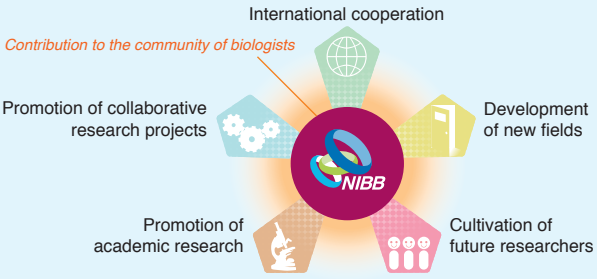
Members of the Advisory Committee for Programming and Management (terms of April 2019 to March 2021)

Non-NIBB members	HANASHIMA, Carina	Professor, Waseda University
	HIRAOKA, Yasushi ##	Professor, Osaka University
	KITANO, Jun	Professor, National Institute of Genetics
	KOHCHI, Takayuki	Professor, Kyoto University
	KUROIWA, Asato	Professor, Hokkaido University
	SATAKE, Akiko	Professor, Kyushu University
	SIOMI, Mikiko	Professor, The University of Tokyo
	SUGIMOTO, Asako	Professor, Tohoku University
	YAMAMOTO, Takashi	Professor, Hiroshima University
	YOSHIMURA, Takashi	Professor, Nagoya University
NIBB members	AOKI, Kazuhiro	Professor, ExCELLS and National Institute for Basic Biology
	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	HIGASHIJIMA, Shin-ichi	Professor, ExCELLS and National Institute for Basic Biology
	KAWAGUCHI, Masayoshi #	Professor, National Institute for Basic Biology
	MINAGAWA, Jun	Professor, National Institute for Basic Biology
	NIIMI, Teruyuki	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, ExCELLS and National Institute for Basic Biology
	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. 'Collaborative Research Projects for Integrative Genomics' and 'Collaborative Research Projects for Integrative Bioimaging' are 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	2017	2018	2019
Priority collaborative research projects	2	1	1
Collaborative research projects for model organisms and technology development	2	2	2
Individual collaborative research projects	51	57	60
Collaborative research projects for integrative genomics	62	67	66
Collaborative research projects for integrative bioimaging	28	23	22
NIBB workshops	3	2	3
Collaborative experiments using the Large Spectrograph	9	9	9
Support for NIBB training courses	0	1	0
Collaborative research projects for bioresource preservation technology development	12	18	14
Total	169	180	177

NIBB Core Research Facilities

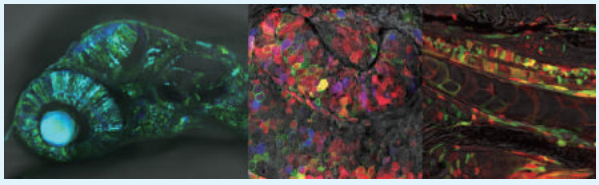
The NIBB Core Research Facilities support research at NIBB and also act as an intellectual hub to promote collaboration between NIBB and other academic institutions. They consist of three facilities that are developing and providing state-of-the-art technologies through functional genomics, bioimaging and bioinformatics (p. 82).

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. 106). 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 professor and specially appointed associate professor, 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 (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 of the Inter-University Bio-Backup Project (IBBP Center)

To prevent damage to important biological resources by natural disasters, NIBB established the IBBP Center in 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources used in cutting-edge research (p. 91).

Center for the Development of New Model Organisms

This center was established in 2013 and employs a cross-appointment researcher who developed and refined genome editing techniques in particular in *Pleurodeles waltl* (Iberian ribbed newt) (p. 93) and also taught these techniques in the training course (p. 108).

Advanced Bioimaging Support (ABiS)

ABiS provides an 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 specialized imaging equipment. In 2018, ABiS joined the Global Bioimaging (GBI) network representing the Japanese bioimaging community. ABiS also hosts imaging competitions and training courses (p. 108).

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 Symposium "Imaging and Quantitative Biology" was held in October, 2019 (p. 102).

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" was held jointly with ABiS in February, 2019 (p. 100). The 67th conference "Quest for Orthologs" was hels in July, 2019 (p. 101).

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. International conferences and courses are managed by the International Cooperation Group of the Research Enhancement Strategy Office.

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. Streaming live videos of the development of model organisms successfully attract many accesses. Our triannual open campus event was held in October, 2019 at which we welcomed nearly 3,000 local citizens (p. 111). 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. 96).

Development of New Fields of Biology

Bioimaging

NIBB aims to maximize the application of modern light microscopes and biophotonic probes for real time visualization of biological phenomena and to develop new imaging techniques. As part of our collaborative work with EMBL, NIBB introduced a DSLM, which is effective for the three-dimensional observation of living organisms, and has developed an improved model using two-photon optics (p. 77). 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 13th Forum "Behavioral and Recognition Research upon the Platform of Vision and Color" was held in February, 2019 (p. 106). A training course in bioimage analysis was also held in December, 2019 (p. 107).

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

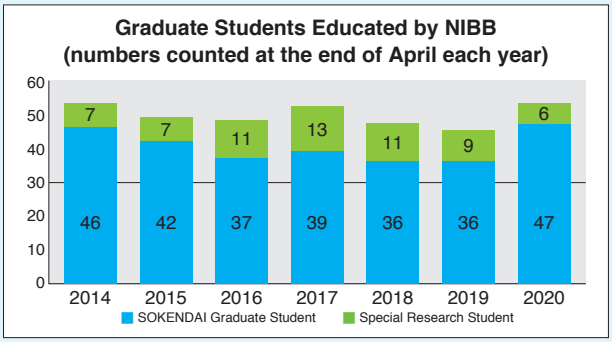
NIBB constitutes the Department of Basic Biology in the School of Life Science of SOKENDAI (Graduate University for Advanced Studies). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree. Graduate students enrolled 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. 110).

Support for young researchers is managed by the Young Researcher Support Group of the Research Enhancement Strategy Office (p. 98).



Personnel changes from January 2019 to March 2020*

Newly assigned to NIBB

Name	Position	Research Unit	Date
AGATA, Kiyokazu	Director General	Laratory of Regeneration Biology	April 1, 2019
SHIGENOBU, Shuji	Professor	Laboratory of Evolutionary Genomics	April 1, 2019
TANIMOTO, Masashi	Assistant Professor	Division of Behavioral Neurobiology	April 1, 2019
SHIKATA, Hiromasa	Assistant Professor	Division of Plant Environmental Responses	April 1, 2019
KOBAYASHI, Taisuke	NIBB Research Fellow#	Laboratory of Neurophysiology	April 1, 2019
YOKE, Hiroshi	NIBB Research Fellow#	Laboratory for Spatiotemporal Regulations	April 1, 2019
SHINOZUKA, Takuma	NIBB Research Fellow#	Division of Molecular and Developmental Biology	April 1, 2019
LIU, Meng	NIBB Research Fellow#	Division of Symbiotic Systems	October 1, 2019
GOTO, Yuhei	Assistant Professor	Division of Quantitative Biology	November 1, 2019
MANO, Hiroaki	Specially Appointed Assistant Professor	Division of Evolutionary Biology	November 1, 2019
KANAI, Masatake	Specially Appointed Assistant Professor	Laboratory of Organelle Regulation	February 1, 2020

Newly affiliated with other universities and institutes

Name	New Affiliation	Position	Date
NODA, Masaharu	Tokyo Institute of Technology	Specially Appointed Professor	April 1, 2019
SHINTANI, Takafumi	Tokyo Institute of Technology	Specially Appointed Associate Professor	April 1, 2019
MATSUDA, Takashi	Tokyo Institute of Technology	Specially Appointed Assistant Professor	April 1, 2019
HIYAMA, Takeshi	Okayama University	Lecturer	April 1, 2019
SUZUKI, Makoto	Hiroshima University	Assistant Professor	April 1, 2019
TAMADA, Yosuke	Utsunomiya University	Associate Professor	August 1, 2019
ANSAI, Satoshi	Tohoku University	Assistant Professor	November 1, 2019
MIYANARI, Yusuke	Kanazawa University	Associate Professor	April 1, 2020
MURATA, Takashi	Kanagawa Institute of Technology	Professor	April 1, 2020

* Changes in professors, associate/ assistant professors, and NIBB research fellows.
# NIBB Research Fellows were reappointed as Specially Appointed Assistant Professors on April 1, 2020.

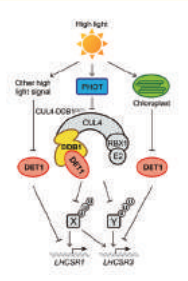
Awardees from January 2019 to March 2020

Name	Position	Award
KITADATE, Yu	Assistant Professor	NINS Young Researcher Award
HASEBE, Mitsuyasu	Professor	Golden Spore Award 2019 (International Molecular Moss Science Society)

Selected Press Releases from NIBB (Jan. 2019 to Mar. 2020)

Press releases on a paper whose first and corresponding authors both have affiliation in NIBB

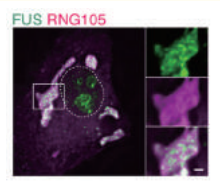
1 January, 2019



Elucidating algal protection mechanisms against excessively strong light and its core component, E3 ubiquitin ligase, prevailing from plants to animals

Aihara, Y., Fujimura-Kamada, K., Yamasaki, T., and Minagawa, J. (2019). Algal photoprotection is regulated by the E3 ligase CUL4-DDB1DET1. Nat. Plants *5*, 34-40. doi: 10.1038/s41477-018-0332-5

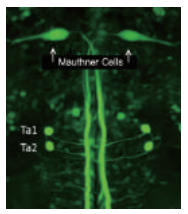
17 January, 2019



The characterization of a group of proteins necessary for intracellular compartmentalization not performed by the cell membrane: Formation of liquid and solid-like RNA granules by proteins related to long-term memory, ALS and dementia

Shiina, N. (2019). Liquid- and solid-like RNA granules form through specific scaffold proteins and combine into biphasic granules. J. Biol. Chem. *294*, 3532-3548. doi: 10.1074/jbc.RA118.005423

18 January, 2019



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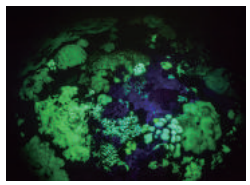

Green fluorescence from reef-building corals attracts symbiotic algae

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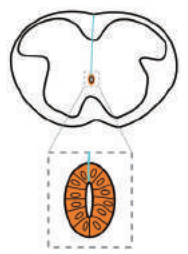

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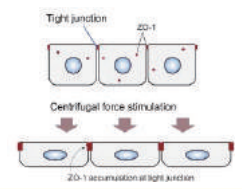

Elucidating cellular responses to force: Mechanical force induces phosphorylation-mediated signaling that underlies tissue response and robustness in *Xenopus* embryos

Hashimoto, Y., Kinoshita, N., Greco, T.M., Federspiel, J.D., Jean Beltran, P.M., Ueno, N., and Cristea, I.M. (2019). Mechanical force induces phosphorylation-mediated signaling that underlies tissue response and robustness in *Xenopus* embryos. Cell Syst. *8*, 226-241. doi: 10.1016/j.cels.2019.01.006

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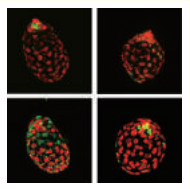

The mechanism underlying the temporal suspension (diapose) of the development of mammalian embryos: The timing of suspension and resumption of development differs depending on embryonic region and the individual cell

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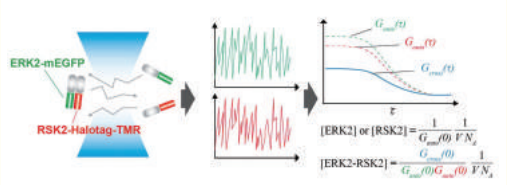

Single-living-cell quantification of the concentrations and dissociation constants of endogenous proteins by genome editing and microscopic techniques

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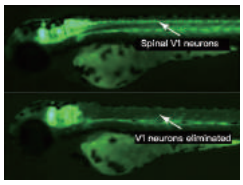

Identifying the neural mechanisms that inhibit slow muscle activity during fast swimming in fish

Kimura, Y., and Higashijima, S.-I. (2019). Regulation of locomotor speed and selection of active sets of neurons by V1 neurons. Nat. Commun. *10*, 2268. doi: 10.1038/s41467-019-09871-x

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A novel mechanism for increasing the quality and quantity of oils in soybeans

Kanai, M., Yamada, T., Hayashi, M., Mano, S., and Nishimura, M. (2019). Soybean (Glycine max L.) triacylglycerol lipase GmSDP1 regulates the quality and quantity of seed oil. Sci. Rep. *9*, 8924. doi: 10.1038/s41598-019-45331-8

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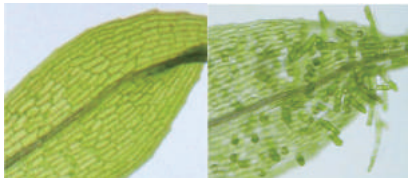

Finding of STEMIN (STEM CELL INDUCING FACTOR) for feasible reprogramming in plants

Ishikawa, M., Morishita, M., Higuchi, Y., Ichikawa, S., Ishikawa, T., Nishiyama, T., Kabeya, Y., Hiwatashi, Y., Kurata, T., Kubo, M., Shigenobu, S., Tamada, Y., Sato, Y., and Hasebe, M. (2019). Physcomitrella STEMIN transcription factor induces stem cell formation with epigenetic reprogramming. Nat. Plants *5*, 681-690. doi: 10.1038/s41477-019-0464-2

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The discovery of a new function of the gene (CONSTANS) which controls the timing of flower formation in flowering plants: CONSTANS controlls photoprotection in green algae

Tokutsu, R., Fujimura-Kamada, K., Matsuo, T., Yamasaki, T., and Minagawa, J. (2019). The CONSTANS flowering complex controls the protective response of photosynthesis in the green alga *Chlamydomonas*. Nat. Commun. *10*, 4099. doi: 10.1038/s41467-019-11989-x

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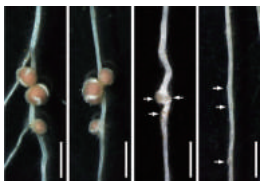

Recruitment of a lateral root developmental pathway into root nodule formation of legumes

Soyano, T., Shimoda, Y., Kawaguchi, M., and Hayashi, M. (2019). A shared gene drives lateral root development and root nodule symbiosis pathways in *Lotus*. Science *366*, 1021-1023. doi: 10.1126/science.aax2153

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The determination of the 3d structure of the supercomplex between the light-harvesting complex II and the photosystem II: The full picture of a huge light-harvesting machine with a molecular weight of 1.66 million

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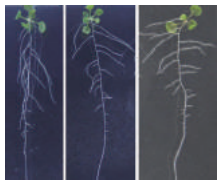

Discovery of a new factor informing the plant root of the direction of gravity: A mechanism that makes auxin flow toward the direction of gravity

Furutani, M., Hirano, Y., Nishimura, T., Nakamura, M., Taniguchi, M., Suzuki, K., Oshida, R., Kondo, C., Sun, S., Kato, K., Fukao, Y., Hakoshima, T., and Morita, M.T. (2020). Polar recruitment of RLD by LAZY1-like protein during gravity signaling in root branch angle control. Nat. Commun. *11*, 76. doi: 10.1038/s41467-019-13729-7

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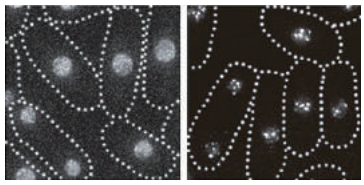

Discovery of a mechanism that selectively eliminates the transcripts of meiotic genes necessary for the formation of reproductive cells

Shichino, Y., Otsubo, Y., Yamamoto, M., and Yamashita, A. (2020). Meiotic gene silencing complex MTREC/NURS recruits the nuclear exosome to YTH-RNA-binding protein Mmi1. PLoS Genet. *16*, e1008598. doi: 10.1371/journal.pgen.1008598

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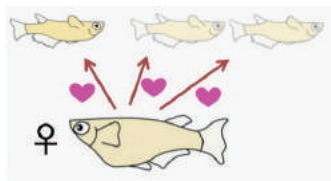

The oxytocin (love hormone) controls mate choice in medaka: Oxytocin works in a contrary manner between males and females

Yokoi, S., Naruse, K., Kamei, Y., Ansai, S., Kinoshita, M., Mito, M., Iwasaki, S., Inoue, S., Okuyama, T., Nakagawa, S., Young, L.J., and Takeuchi, H. (2020). Sexually dimorphic role of oxytocin in medaka mate choice. Proc. Natl. Acad. Sci. USA *117*, 4802-4808. doi: 10.1073/pnas.1921446117

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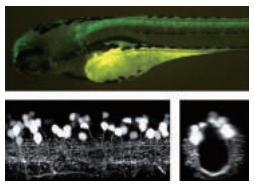

The importance of the commissural inhibitory neurons that control coordinated left-right body movement in larval zebrafish

Satou, C., Sugioka, T., Uemura, Y., Shimazaki, T., Zmarz, P., Kimura, Y., and Higashijima, S.-I. (2020). Functional diversity of glycinergic commissural inhibitory neurons in larval zebrafish. Cell Rep. *30*, 3036-3050. doi: 10.1016/j.celrep.2020.02.015

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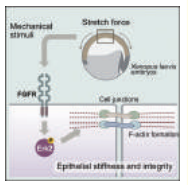

Control mechanism of force-induced cell-to-cell adhesion

Kinoshita, N., Hashimoto, Y., Yasue, N., Suzuki, M., Cristea, I.M., and Ueno, N. (2020). Mechanical stress regulates epithelial tissue integrity and stiffness through the FGFR/Erk2 signaling pathway during embryogenesis. Cell Rep. *30*, 3875-3888. doi: 10.1016/j.celrep.2020.02.074

Division of Cellular Dynamics

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Membrane traffic between single membrane-bounded organelles plays an integral role in various activities in eukaryotic cells. Recent comparative genomics have indicated that membrane trafficking pathways are diversified among eukaryotic lineages, which are 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. This 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. Comparisons of these protein families' organizations among plant lineages, followed by functional analyses of each gene product in *A. thaliana* and *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.

1-1 Analysis of the liverwort-specific organelle: the oil body

Through analyses of SNARE members in *M. polymorpha*, a member of the SYP1 group was localized to the membrane of the oil body, an organelle specific to liverworts, whose origin and biogenesis remain unclear.

Furthermore, we discovered an oil body formation master regulator, whose molecular function is currently being investigated. We also characterized the membrane trafficking pathway responsible for oil body biogenesis, and found that the trafficking pathway to the oil body should be a redirected secretory pathway.

The morphology and distribution pattern of the oil body, *e.g.* its shape, color, number, and a density of oil body cells in tissues diverge among liverwort species, which, therefore, are regarded as important features for taxonomical classification in liverworts. However, molecular mechanisms of oil body morphogenesis remain to be determined. We have successfully isolated some mutants defective in oil body morphogenesis in *M. polymorpha* (Figure 1), which would lead to identification of unknown factors involved in oil body biogenesis.

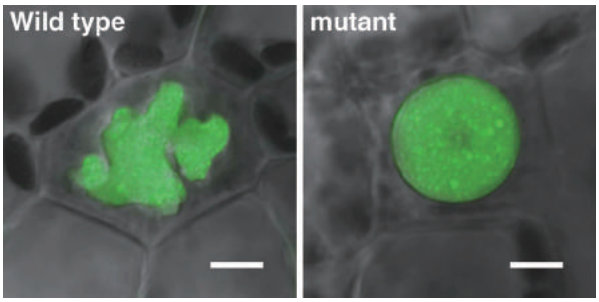

Figure 1. Oil bodies in wild-type and mutant *M. polymorpha*. The oil body exhibits complex morphology in the wild-type plant, whereas spherical in the mutant. Bars = 5 μm.

1-2 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 the 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, which is a process distinctly regulated from non-plant systems (for example, Takemoto *et al.*, 2018).

Defective vacuolar SNARE functions affect both vacuolar transport and morphology. The *sgr3-1* (*shoot gravitropism3*) mutant was isolated as a mutant deemed defective in shoot gravitropism. This resulted from a point mutation in *SYP22/VAM3*, which is one of the SNARE proteins residing on the vacuole and active in vacuolar transport. The *sgr3-1* mutant exhibits abnormal vacuolar morphology, although vacuolar transport is not markedly affected in this mutant. We also found that machinery components for homotypic vacuolar membrane fusion including VAMP71, SYP22, and the tethering HOPS complex were accumulated at specific domains in the vacuolar membrane in *sgr3-1*. These results suggested that vacuolar membrane homotypic fusion is specifically affected by the *sgr3-1* mutation.

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

2-1 Reorganization of the membrane trafficking system during pathogenic fungus invasion

Membrane traffic also plays important roles in plant-microbe interaction. Phosphoinositides, which are minor phospholipids in endomembranes, function within membrane traffic and signaling. Systematic observation of phosphoinositides during the invasion of the pathogenic fungi, Colletotrichum, revealed the strong accumulation of PtdIns(4,5)P2 in the extra-invasive hyphal membrane (EIHM) (Figure 2). During Colletotrichum infection, an exocytic factor was recruited to the EIHM, but endocytic factors were eliminated. Furthermore, overexpression of Arabidopsis PIP5K, which catalyzes PtdIns4P into PtdIns(4,5)P2, increased the Colletotrichum invasion, suggesting that the pathogenic fungi, Colletotrichum, could modify PtdIns in the EIHM in the successful infection (Shimada *et al*., 2019).

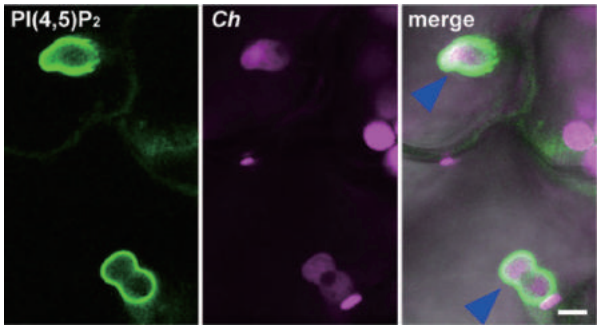

Figure 2. Accumulation of PtdIns(4,5)P2 in the EIHM formed by Colletotrichum (*Ch*). Bar = 5 μm. (Shimada *et al*., 2019)

2-2 Membrane trafficking in plant gametogenesis

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

Cytokinesis in land plants is achieved by the re-direction of the secretory pathway. As such, KNOLLE/SYP111 plays important roles in membrane fusion in the forming of cell plates in *A. thaliana* somatic cells. Conversely, no deleterious effects on gametogenesis have been reported regarding mutations in KNOLLE. We found that KNOLLE and other SYP1 members were highly expressed during cytokinesis in gametogenesis (Figure 3). Mutant analyses of *syp1* members also supported that KNOLLE and other SYP1 regulate cytokinesis during gametogenesis in *A. thaliana* .

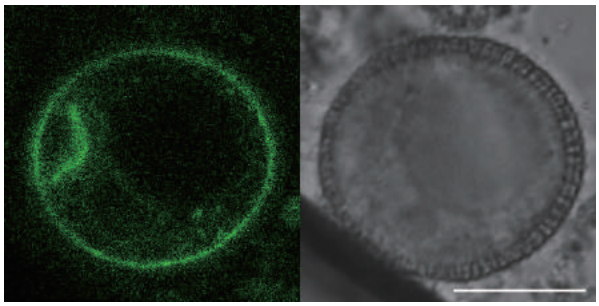

Figure 3. Expression and subcellular localization of GFP-KNOLLE during pollen mitosis I. GFP-KNOLLE accumulates at the cell plate. Bar = 10 μm. t

Distinct from seed plants, basal land plants including *M. polymorpha* utilize the spermatozoid with two (or more) motile flagella as the male gamete during sexual reproduction. We visualized the spermatozoid formation process, especially spermiogenesis, using fluorescently-tagged organelle markers in *M. polymorpha*. 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 endosome, vacuole, and autophagosome, was transiently increased during this process. To reveal the molecular mechanisms of cytoplasm removal and organelle remodeling, we have established the analytical tools of autophagy in *M. polymorpha* (Norizuki *et al*., 2019). *M. polymorpha* possesses core machineries of autophagy with lower degrees of redundancy. The mutations in Mp*ATG5* and Mp*ATG7*, which are key factors for autophagosome formation, affected the transportation of cytosolic components to the vacuole for degradation (Figure 4).

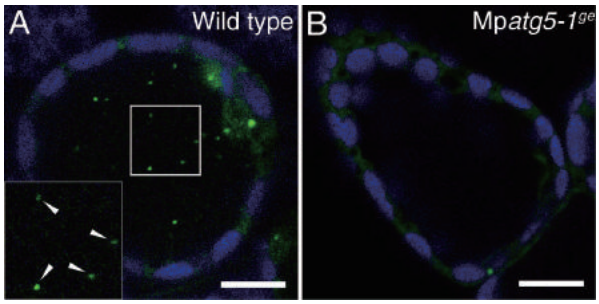

Figure 4. Establishment of analytical tools for the autophagy study in *M. polymorpha*. YFP-tagged MpATG8a is targeted to vacuole in wild type (A), but not in the Mp*atg5-1*^{ge} mutant (B). Bars = 10 μm. (Norizuki *et al*., 2019).

Autophagy-defective mutants exhibited defects regarding cytoplasm removal, spermatozoid motility, and fertility. 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 (Figure 5).

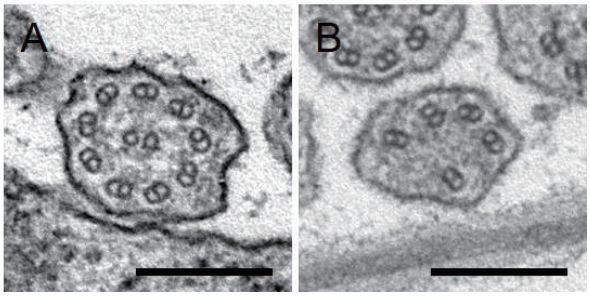

Figure 5. Transverse sections of flagella in wild-type (A) and mutant (B) spermatids. The microtubule-based "9 + 2" axoneme structure is severely compromised in the mutant. Bars = 200 nm.

2-3 Functions of ANTH-domain proteins in plant physiology

AP180 N-terminal homology domain-containing proteins (ANTH proteins) are thought to act as adaptors bridging the clathrin coat and cargo proteins during clathrin-coated vesicle formation. ANTH proteins exhibit remarkable expansion during land plant evolution, and we examined how this protein family has been functionally diversified in *A. thaliana*. We found that a pair of 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*, whereas another receptor kinase PRK6 acting in pollen tube guidance is not affected (Muro *et al*., 2018). Now we are looking for PICALM members regulating localization of PRK6 and other receptors in Arabidopsis pollen tube.

We also found that another paralogous set of PICALM proteins is required for retrieving secretory SNARE proteins from the plasma membrane (under revision), which itself is required for normal vegetative development. These lines of evidence indicated that ANTH proteins are functionally differentiated, which in turn underpin various physiological processes in *A. thaliana*.

Publication List:

〔Original papers〕

- 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, B.-H., and Jiang, L. (2019). A whole-cell electron tomography model of vacuole biogenesis in *Arabidopsis* root cells. Nat. Plants *5*, 95-105. doi: 10.1038/s41477-018-0328-1
- D'Ippolito, R.A., Minamino, N., Rivera-Casas, C., Cheema, M.S., Bai, D.L., Kasinsky, H.E., Shabanowitz, J., Eirin-Lopez, J.M., Ueda, T., Hunt, D.F., and Ausió, J. (2019). Protamines from liverwort are produced by post-translational cleavage and C-terminal di-aminopropanelation of several male germ-specific H1 histones. J. Biol. Chem. *294*, 16364-16373. doi: 10.1074/jbc.RA119.010316
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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, thus leading to an adaptive response (Figure 1). This cellular information processing is mainly controlled by intracellular signal transduction, which is comprised of a series of chemical reactions such as protein-protein interaction and protein phosphorylation. Dysregulation of cell signaling by gene mutation is widely known to result in pathological diseases, such as malignant tumors.

The intracellular signaling pathway has been extensively studied over the past few decades. As a result, many proteins and regulations have been identified, which has resulted in an increase in the pathway's complexity. Computer-assisted simulation is one of the most promising approaches in the understanding of the signal transduction pathway as a system. Indeed, chemical and physical reactions constituting the signal transduction can be described by a set of ordinary differential equations and can be solved numerically by computers. Several signaling pathway simulation models have been reported to date. However, most of the kinetic parameters utilized for these simulation models have not been measured experimentally, but have been assumed by being simply arbitrarily determined. Consequently, there are substantial differences in the kinetic parameters among 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 that enable us to (1) visualize, (2) quantify, and (3) manipulate intracellular signaling pathways.

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 of a donor fluorophore is transferred to a nearby acceptor fluorophore. Taking advantage of this principle, FRET-based biosensors allowed us to detect PKA, ERK, Akt, JNK, PKC, and S6K's kinase activity in living cells with a high temporal and spatial resolution (Komatsu N, Mol. Biol. Cell, 2011). By using a FRET biosensor, we have been able to reveal the role played by ERK activation dynamics in cell proliferation (Aoki K, Mol Cell, 2013) and collective cell migration (Aoki K, Dev Cell, 2017).

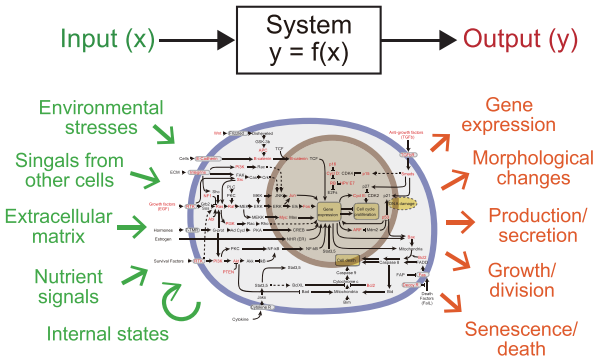

Figure 1. Information processing by intracellular signaling devices and networks.

Although FRET biosensors enable us to monitor cell signaling events, FRET imaging is not suitable for multiplexed imaging, because the biosensors are composed of two different fluorophores in many cases. To circumvent this limitation, we are currently developing single-fluorophore based biosensors. We recently developed a red-fluorescence dopamine (DA) reporter. DA, a neuromodulator, is involved in many neuronal functions. To monitor DA in living cells

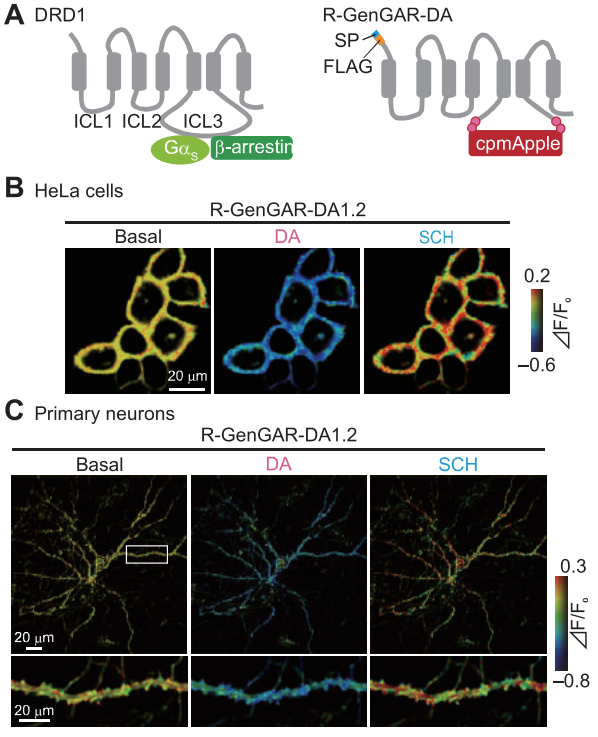

Figure 2. Development of a genetically encoded red fluorescent dopamine (DA) reporter. (A) Schematic representation of a dopamine receptor, DRD1 (left), and Red DA reporter, R-GenGAR-DA. (B and C) HeLa cells (B) or mouse primary hippocampal neurons (C) expressing R-GenGAR-DA were stimulated with DA, followed by a DRD1 antagonist, SCH.

and animals, we designed the red-fluorescent DA reporter with circular-permutated mApple (cpmApple), a red fluorescent protein; cpmApple was inserted into the third intracellular loop of a DA receptor, DRD1 (Figure 2A). The linker sequences between DRD and cpmApple were optimized by random mutagenesis and subsequent live-cell screening. Finally, we picked up the best performance reporter, and named the red genetically encoded GPCR activation reporter for DA, R-GenGAR-DA. The red fluorescence intensity decreased in response to DA, and returned to the basal level upon a DRD1 antagonist treatment (Figure 2B and 2C). In addition, by using previously reported green fluorescence norepinephrine (NE) reporter, we succeeded in simultaneously visualizing DA and NE in living neurons (Nakamoto C, Goto Y, *et al*., bioRxiv, 2020).

II. Quantification of cell signaling and physical parameters

Kinetic parameters such as protein concentration and dissociation constant, Kd, have been measured by conventional *in vitro* biochemical analyses. However, some kinetic parameters could 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 to 2 (Sadaie W, *et al*., MCB, 2014). Therefore, it is critical to measure kinetic parameters in living cells. To this end, we combined CRISPR/Cas9-mediated genome editing techniques with quantitative fluorescence imaging techniques.

First, we developed an efficient knock-in system, and tagged endogenous proteins with fluorescent proteins by Cas9-mediated genome editing (Figure 3A). Next, we quantified their concentrations and Kd value by fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) (Figure 3B). These analyses revealed temporal changes in Kd values of the binding between ERK2 and RSK2 in response to EGF. Our approach provides a robust and efficient method for quantifying endogenous protein concentrations and dissociation constants in living cells (Komatsubara AT, Goto Y, *et al*., JBC, 2019).

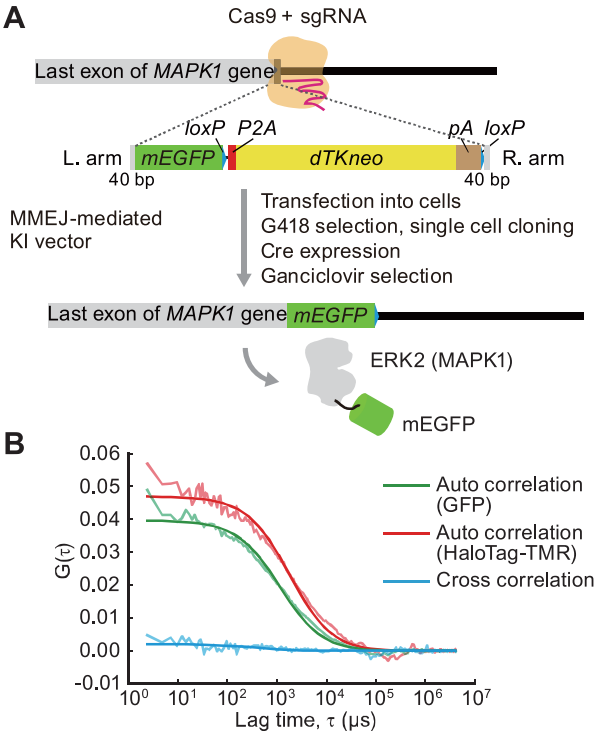

Figure 3. Quantification of protein concentration and dissociation constant of endogenous proteins. (A) Schematic illustration of gene knock-in with an MMEJ-mediated KI vector at MAPK1 locus. (B) Auto- and cross-correlation functions of ERK2-mEGFP and RSK2-HaloTag. *mEGFP* and *HaloTag* genes were knocked-in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

III. Manipulation of cell signaling

Artificial manipulation of biochemical networks are 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 beneficial in terms of temporal and spatial manipulations. The photo-responsive proteins derived from fungi, cyanobacteria, plants, and modified fluorescent proteins are used in the LID system.

Among these, we focus on the phytochrome B (PhyB)-PIF LID system. Upon red-light illumination, PhyB binds to PIF, and the PhyB-PIF complex dissociate from each other by far-red light exposure (Figure 4A). The reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system, because other LID systems can only control association or dissociation by light. One drawback is that covalent attachment of a chromophore, phycocyanobilin (PCB), is required for the light-responsive function of PhyB-PIF. To overcome this issue, we have developed a method for biosynthesis of PCB in mammalian cells by introduction of the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into mitochondria (Uda Y, *et al*., PNAS, 2017) (Figure 4A). Recently, we further improved the system for PCB synthesis, which allowed establishing stable cell lines synthesizing PCB and light-induced control of protein localization at the cell population level (Figure 4B and 4C).

To take full advantage of this, we applied the genetically encoded PCB synthesis system to *S. pombe* and *C. elegans*, both of which are incapable of having purified PCB delivered to them. As we expected, the expression of *HO1*, *PcyA*, *Fd*, and *Fnr* genes induced PCB biosynthesis in *S. pombe*

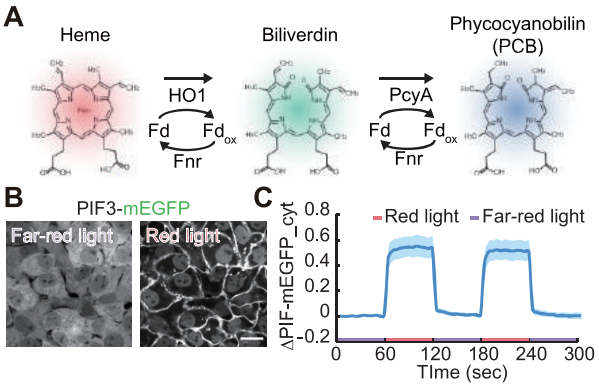

Figure 4. PhyB-PIF light-inducible dimerization (LID) system. (A) The metabolic pathway of phytochrome chromophores, PCB. (B and C) Light-induced translocation of PIF3-mEGFP to the plasma membrane, where PhyB is localized (B). Membrane translocation of PIF3-mEGFP is quantified (C).

and *C. elegans*. Using this system, we developed optogenetic control of the cell cycle in two ways: the Opto-G2/M checkpoint triggered G2/M cell cycle arrest in response to red light, and Opto-SAC induced a spindle assembly checkpoint (SAC) in response to red light and then quickly released the SAC by far-red light (Figure 5). The genetically encoded system of PCB synthesis would 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.

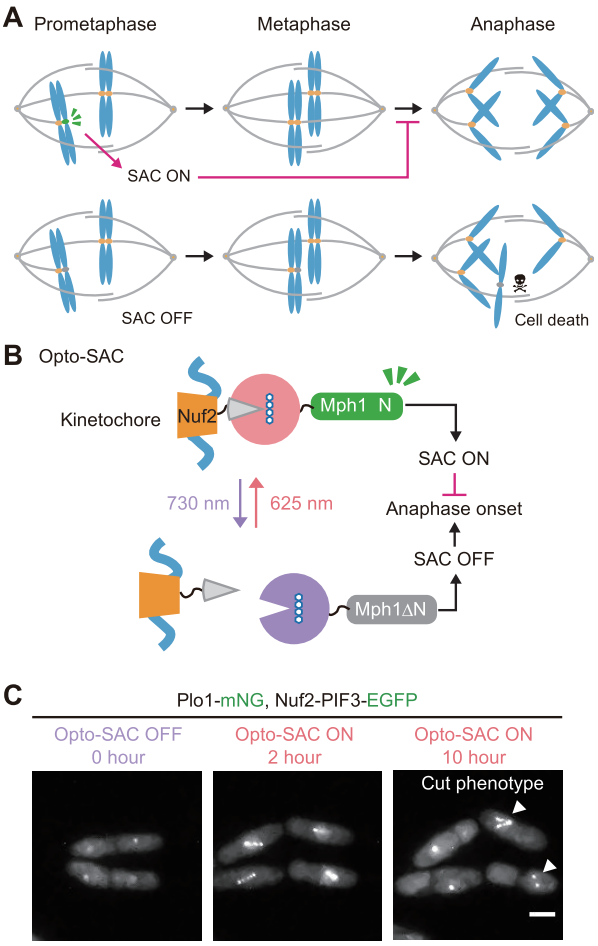

Figure 5. Optogenetic control of spindle assembly checkpoint (Opto-SAC). (A) Schematic of the spindle assembly checkpoint (SAC), which ensures the faithful chromosome segregation and anaphase onset by monitoring kinetochore-microtubule attachment. (B) Schematic of the design of Opto-SAC. Mph1ΔN, which lacks the kinetochore binding domain, is fused with PhyB (PhyB-mph1ΔN). Endogenous Nuf2 is fused with PIF3 (nuf2-PIF3) as a kinetochore localizer. Upon red light illumination, PhyB-Mph1ΔN is recruited to kinetochores through the binding to Nuf2-PIF3, and SAC turns ON. Far-red light exposure induces dissociation of PhyB-Mph1ΔN from kinetochores, leading to the inhibition of activated SAC. (C) Representative cells undergoing a failure of mitosis under long-term metaphase arrest by Opto-SAC.

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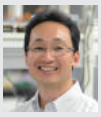
DIVISION OF CHROMATIN REGULATION



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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 said 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* (*S. pombe*), in addition to 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

1-1 H3K14 ubiquitylation promotes H3K9 methylation for heterochromatin assembly

In eukaryotic cells, the assembly of higher-order chromatin structures, known as heterochromatin, plays an important role in diverse chromosomal processes. Heterochromatin assembly is intimately associated with changes in post-translational histone-tail modifications. Histone H3-lysine 9 methylation (H3K9me), a hallmark of heterochromatin structure, is catalyzed by SUV39H-family histone methyltransferases, and functions as a binding site for recruiting heterochromatin protein 1 (HP1) family proteins. In the fission yeast *S. pombe*, Clr4, a homolog of mammalian SUV39H, plays a central role in heterochromatin assembly. Clr4 forms a multi-protein complex called the Clr4 methyltransferase complex (CLRC) with Cul4, Rik1, Raf1, and Raf2. Cul4, Rik1, and Raf1 display a strong structural resemblance to the conserved CUL4-DDB1-DDB2 E3 ubiquitin ligase, and it has been demonstrated that the CLRC exhibits ubiquitin ligase activity *in vitro*. However, whether the CLRC acts as an E3 ubiquitin ligase *in vivo* and how ubiquitylation modulates Clr4's activity remains unclear.

To identify the physiological substrate(s) ubiquitylated by CLRC, we affinity purified the CLRC (Figure 1A) and performed an *in vitro* ubiquitylation assay using histones as candidate substrates (Figure 1B). We discovered that the affinity-purified CLRC specifically ubiquitylates recombinant histone H3, and mass spectrometric and mutation analyses revealed that H3-lysine 14 (H3K14) is the preferred target of the complex (Figure 1C).

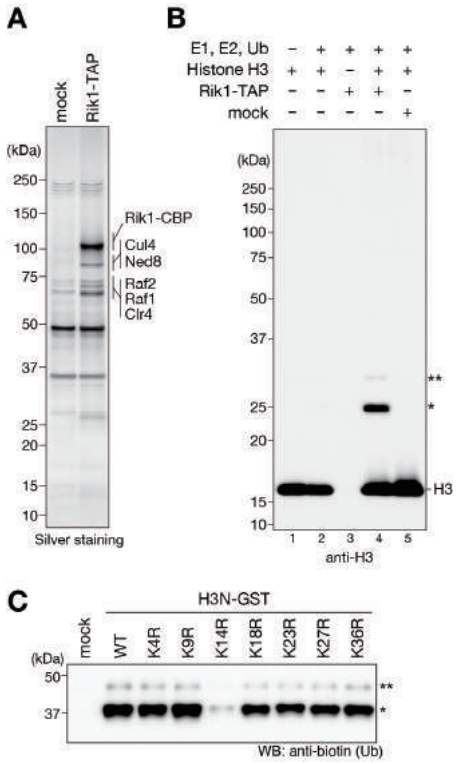

Figure 1. Histone H3 lysine 14 is ubiquitylated *in vitro* by CLRC. (A) Purified TAP-tagged Rik1-containing complexes analyzed by SDS-PAGE and silver staining. Proteins identified by LC-MS/MS are indicated on the right. (B) *In vitro* ubiquitylation assays using biotinylated ubiquitin, purified CLRC, and recombinant histone H3 as the substrate. Proteins were analyzed by Western blotting using the indicated antibodies. Asterisks indicate ubiquitylated histone H3 species. (C) Ubiquitylation assay using biotinylated ubiquitin and recombinant wild-type H3N-GST (WT) and arginine-substituted H3N-GST mutants as substrates. Proteins were analyzed by Western blotting. Asterisks indicate ubiquitylated H3N-GST proteins.

We next sought to assess the presence of K14-ubiquitylated H3 (H3K14ub) *in vivo*. We performed chromatin immunoprecipitation (ChIP) assays using antibodies against H3K9me2 and performed LC-MS/MS analyses. We confirmed that H3K14ub was detected exclusively in the H3K9me2-associated heterochromatin.

We then examined the possible connection between H3K14ub and H3K9me. To test whether H3K14ub modulates H3K9me, we performed *in vitro* histone methyltransferase (HMTase) assays using recombinant Clr4 and ubiquitylated H3N-GST as a substrate (Figure 2A). H3N-GST was first subjected to an *in vitro* ubiquitylation assay using affinity-purified CLRC. After the reaction, the resultant H3N-GST was purified and subjected to an *in vitro* HMTase assay. We found that even though only 10-15% of the H3N-GST was ubiquitylated by the CLRC under our assay condi-

tions and the rest remained unmodified, Clr4 exclusively methylated the ubiquitylated H3N-GST (Figure 2B). These results suggest that the CLRC-mediated H3 ubiquitylation promotes H3K9me by Clr4 and that H3 ubiquitylation is intimately linked to the establishment and/or maintenance of H3K9me. These findings also demonstrate a cross-talk mechanism between histone ubiquitylation and methylation that is involved in heterochromatin assembly (Figure 2C).

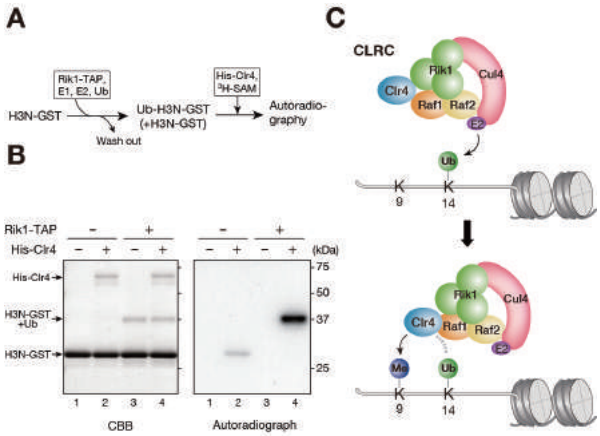

Figure 2. H3K14 ubiquitylation promotes H3K9 methylation. (A) Experimental scheme. (B) *In vitro* HMTase assays. Recombinant H3N-GST pre-ubiquitylated by the CLRC was purified and used in the HMTase assay with 6□His-tagged recombinant Clr4 (His-Clr4). Proteins were analyzed by SDS-PAGE and Coomassie Brilliant Blue (CBB) staining (left) and autoradiography (right). (C) A cross-talk mechanism between histone ubiquitylation and methylation involved in heterochromatin assembly.

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 (Figure 3A). In addition, chromatin fractionation analyses revealed that hinge region-phosphorylated HP1α was preferentially dissociated from mitotic chromatin (Figure 3B). Furthermore, EMSA assays demonstrated that AURKB-mediated phosphorylation contributed to a decrease in HP1α's DNA-binding activity (Figure 3C). 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.

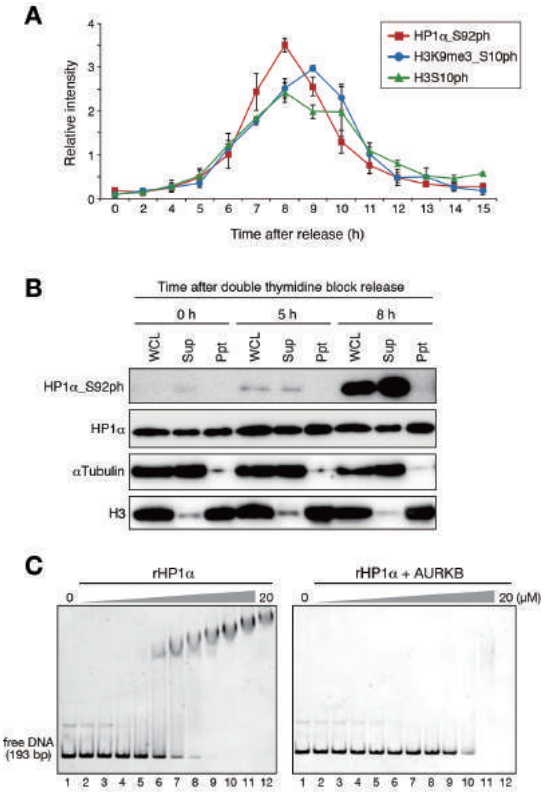

Figure 3. Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding. (A) The levels of HP1α_S92ph, H3K9me3_S10ph, and H3S10ph in synchronized PRE-1 cells. (B) Chromatin fractionation assays were performed using synchronized RPE-1 cells. Whole cell lysates (WCLs) and soluble (Sup) and insoluble chromatin-enriched (Ppt) fractions were resolved by SDS-PAGE and analyzed by immunoblotting. (C) Representative results of EMSAs that were performed with control or phosphorylated HP1α. Various concentrations of HP1α were incubated with 193-bp 601 DNA. The protein-DNA complexes were analyzed by 5% native-PAGE and SYBR Gold staining.

2-2 Identification of Aurora kinase-mediated phosphorylation sites in Swi6/HP1 regulating mitotic chromosome segregation

HP1 is a conserved chromosomal protein that plays important roles in heterochromatin assembly. We previously showed that Swi6, one of two HP1 isoforms in *S. pombe*, is multiplicatively phosphorylated by casein kinase II (CK2) and this phosphorylation is essential for its function in heterochromatin assembly. Several previous studies demonstrated that HP1 is subjected to additional phosphorylation during mitosis. However, the functional importance of HP1's mitotic phosphorylation remains unclear. We revealed that Swi6 mitotic phosphorylation is involved in mitotic chromosomal segregation. Using *E. coli* co-expression system, we demonstrated that Swi6 is phosphorylated by Ark1, a solo Aurora kinase in *S. pombe*, and mutation analyses revealed that serine residues in the N-terminal region of

Swi6 are efficient targets for Ark1. We confirmed that these serine residues are phosphorylated during mitosis *in vivo*. Interestingly, expression of mutant Swi6 containing amino-acid substitutions at the serine residues differentially modulates temperature-sensitive growth of the mutations for Chromosome Passenger Complex (CPC) components. These results suggested that Ark1-mediated Swi6 phosphorylation regulates CPC's function during mitosis.

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

The ciliated protozoan *Tetrahymena themophila* has two functionally distinct nuclei in a single cell: the transcriptionally silent germline micronucleus (MIC) and the transcriptionally active somatic macronucleus (MAC). When the somatic MAC differentiates from the germline MIC during sexual reproduction, approximately 12,000 transposable element (TE)-related sequences are eliminated from the MAC genome. In this process, TEs are heterochromatinized by the pathway related to RNAi/piRNA silencing and the heterochromatin acts as a mark for their elimination (Figure 4A). 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 (Figure 4B) and found several proteins that may act as 'hubs' to assemble heterochromatin components into functionally relevant chromatin structure for DNA elimination. Consequently, we are currently analyzing the roles of these hub proteins both *in vitro* and *in vivo* to understand spatiotemporal regulation of heterochromatin formation and DNA elimination.

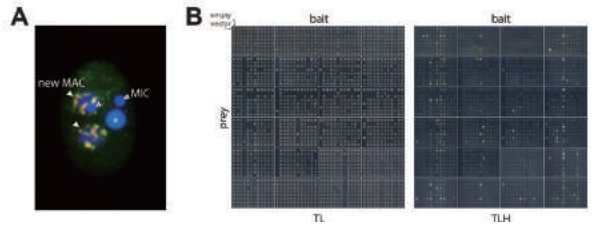

Figure 4. Heterochromatin formation in *Tetrahymena*. (A) *Tetrahymena* cell (at the late stage of sexual reproduction) expressing Pdd1p-mCherry (red) and HPL4-EGFP (red) were counter stained with DAPI (blue). The arrowheads indicate new MAC or MIC and the asterisk indicate old MAC undergoing degradation. (B) Analyses of all possible one-to-one interactions between heterochromatin proteins using yeast two hybrid assay. The yeast strain expressing the Gal4 binding domain fused to a heterochromatin protein (bait) were mated to strains expressing the Gal4 activation domain linked to a heterochromatin protein (prey). Diploid cells were plated on a 48 x 48 matrix on control plates containing all of the auxotrophic requirements (left: without threonine and leucine) and on test plates without histidine (right).

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- Bommi, J.R., Rao, H.B.D.P., Challa, K., Higashide, M., Shinmyozu, K., Nakayama, J.-I., Shinohara, M., and Shinohara, A. (2019). Meiosis-specific cohesin component, Rec8, promotes the localization of Mps3 SUN domain protein on the nuclear envelope. Genes Cells *24*, 94-106. doi: 10.1111/gtc.12653
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- Hiragami-Hamada, K., and Nakayama, J. (2019). Do the charges matter? - Balancing the charges of the chromodomain proteins on the nucleosome. J. Biochem. *165*, 455-458. doi: 10.1093/jb/mvz004

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 exactly 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 currently using mice to research 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, so we can attain a better understanding their relationship to the formation of synapses and neural networks, memory, learning, and behavior.

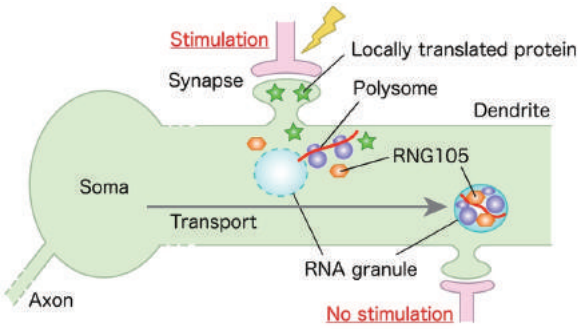

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

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

RNA granules are membrane-less RNA–protein condensates formed by liquid-liquid phase separation (LLPS). They consist of not only dynamic liquid-phase shells but also stable solid-like cores, both of which are thought to function in numerous processes pertaining to 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 the case of these diseases, 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.

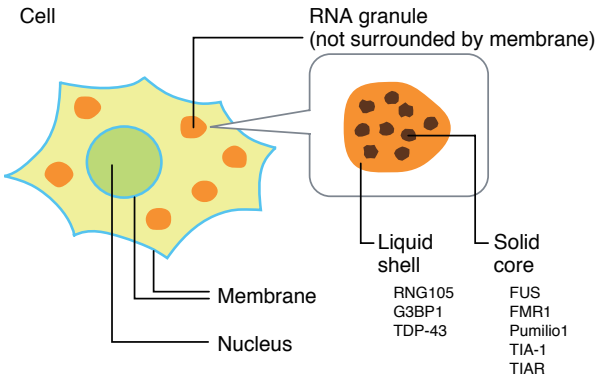

Figure 2. Membrane-less RNA granules contain liquid-like and solid-like substructures. Liquid-phase granules induced by RNG105, G3BP1, and TDP-43, and solid-like granules induced by FUS, FMR1, Pumilio1, TIA-1, and TIAR, combine into biphasic RNA granules.

We expressed 8 kinds of RNA granule scaffold proteins (scaffolds) as GFP and mRFP1-tagged proteins in cultured epithelial cells. Using these cells, we conducted fluorescence microscopy-based morphological and molecular-dynamics analyses (Shiina, J. Biol. Chem., 2019). These analyses demonstrate 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. However, it 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. Furthermore, they also raise questions as to whether the liquid and solid phase properties of RNA granules are also regulated by the scaffold proteins in neurons, and whether such phase properties impact mRNA transport and local translation in neuronal dendrites. We are testing these analyses using mice brains and primary cultured neurons.

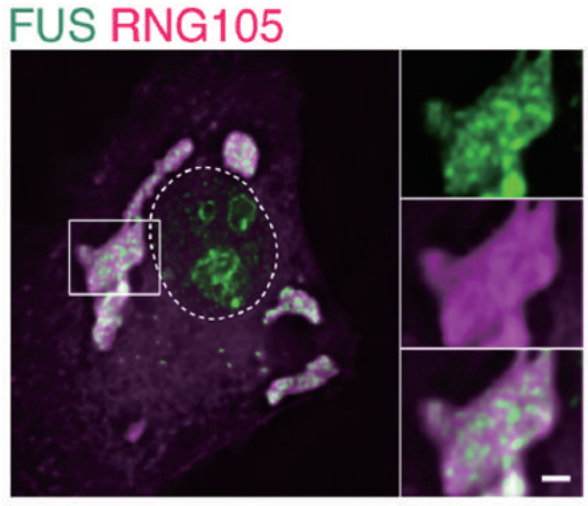

Figure 3. Liquid-type scaffolds (*e.g.* RNG105) and solid-type scaffolds (*e.g.* FUS) combine into the same granules and form individual liquid-like and solid-like substructures. The panels on the right are magnified images of the boxed area. Top: FUS, Middle: RNG105, Bottom: merged. The area surrounded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

II. RNG140 (Caprin2)-mediated translational regulation in eye lens differentiation

RNG105 and RNG140 are paralogous RNA-binding proteins that form distinct RNA granules. RNG105 is highly expressed in neurons and regulates mRNA transport and long-term memory formation (Nakayama *et al*., eLife, 2017), whereas RNG140 is highly expressed in the developing eye lens and plays a role in lens differentiation. Despite RNG140's function in translational regulation, the mechanism and its role within the eye has remained unclear.

We found that RNG140 binds to the translation initiation factor eIF3 using mass spectrometry of RNG140 immunoprecipitates from cultured CHO cells. Reporter translation assay revealed that RNG140 represses translation through mechanisms involving the suppression of eIF3-dependent translation initiation. Comprehensive ribosome profiling demonstrated that overexpression of RNG140 in CHO cells reduces translation of long mRNAs, including those associated with cell proliferation. In fact, RNG140 overexpression slowed the growth rate of CHO cells.

RNG140-mediated translational regulation also operates in the mouse eye, where RNG140 knockout increased the translation of long mRNAs. mRNAs involved in lens differentiation, such as crystallin mRNAs, are short, and were able to escape translational inhibition by RNG140 as well as be translated in differentiating lenses. These findings provide insight into the mechanistic basis of lens cell transition from proliferation to differentiation via RNG140-mediated translational regulation. Moreover, the preference for long mRNAs raised new questions about why and how RNA-binding protein complexes distinguish mRNA lengths in the coordination of proliferation and differentiation.

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

IDRs, which do not form three-dimensional structures, have been revealed to play key roles in LLPS. 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 an IDR and can associate with RNA granules. To investigate the physiological relevance of the IDR associated with NFAR2, we generated NFAR2ΔIDR mice, in which a stop codon was introduced in the exon encoding the IDR in the *Ilf3* gene.

Behavioral analysis demonstrated that NFAR2ΔIDR mice specifically displayed a decrease in fear-conditioned learning, but not in spacial 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 spacial 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.

Publication List:

〔Original paper〕

- Shiina, N. (2019). Liquid- and solid-like RNA granules form through specific scaffold proteins and combine into biphasic granules. J. Biol. Chem. *294*, 3532-3548. doi: 10.1074/jbc.RA118.005423

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- Ohashi, R., and Shiina, N. (2020). Cataloguing and selection of mRNAs localized to dendrites in neurons and regulated by RNA-binding proteins in RNA granules. Biomolecules *10*, 167. doi: 10.3390/biom10020167
- Roy, R., Shiina, N., and Wang, D.O. (2020). More dynamic, more quantitative, unexpectedly intricate: Advanced understanding on synaptic RNA localization in learning and memory. Neurobiol. Learn. Mem. *168*, 107149. doi: 10.1016/j.nlm.2019.107149

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 its 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 this phenomenon 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.

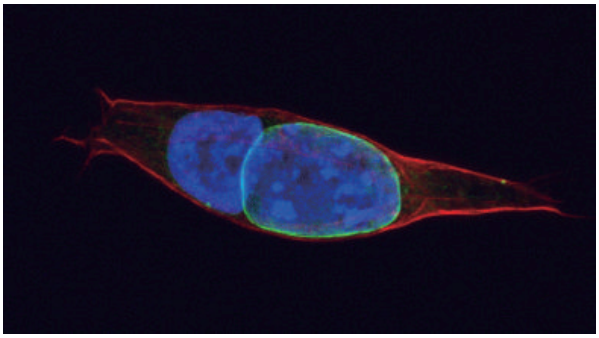

Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus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

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

III. Future 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.

Publication List:

〔Original paper〕

● Kobayashi, M., Ogawa, K., Isobe, M., Nishitani, T., Kamio, S., Fujiwara, Y., Tsubouchi, T., Yoshihashi, S., Uritani, A., Sakama, M., Osakabe, M., and LHD experiment group. Thermal neutron flux evaluation by a single crystal CVD diamond detector in LHD deuterium experiment. J. Instrum. *14*, C09039.

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 utilize various signals generated from environmental changes as being necessary for their survival. The flexibility of plant organelles is the basis 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, as well as 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 during certain developmental stages and when confronted with environmental changes. For example, light induces the 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. In addition to functioning in vegetative tissues such as leaf and root cells, it has been revealed that peroxisomes play essential roles in reproductive processes. Studies using Arabidopsis mutants defective in peroxisomal functions demonstrate that peroxisomes contribute to pollen fertility, pollen tube elongation, and male–female gametophyte recognition. Gene expression, alternative splicing, protein transport, protein degradation and degradation of peroxisomes themselves control these peroxisomal functions.

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 compared to their 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. Based on these results we were able to update the model for protein transport, proliferation and quality control of peroxisomes via autophagy, 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 (Figure 1).

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.

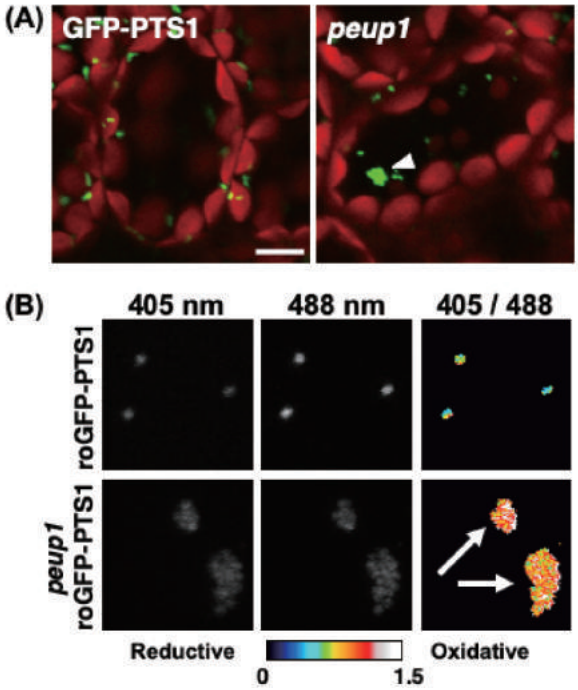

Figure 1. Phenotype of Arabidopsis *peup1* mutant. (A) GFP fluorescence in leaf cells was observed in the parent plant, GFP-PTS1, and *peup1* mutant. *peup1* demonstrates aggregated peroxisomes (arrowhead). Bar indicates 10 μm. (B) Fluorescence intensities from peroxisome-targeted reduction-oxidation-sensitive GFP (roGFP) with two excitation wavelengths were measured, and the 405/488 nm ratio was calculated in the transgenic plants. The 405/488 nm ratio of the peroxisome aggregates in the *peup1* roGFP-PTS1 mutant showed that the peroxisome aggregates were more oxidative than peroxisomes in the wild type.

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 major reserve are proteins. 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.

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 revealed that the extension of *WRINKLED1* (*WRI1*), a tran-

scription factor in fatty acid biosynthesis, expression during the mid-phase 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 the soybean (*Glycine max*. L), we identified four lipases, GmSDP1s, on the oil body membrane. The analyses of GmSDP1s revealed that plant seeds have the mechanism required for the quality control of fatty acids by degrading particular fatty acids in oil bodies (Figure 2).

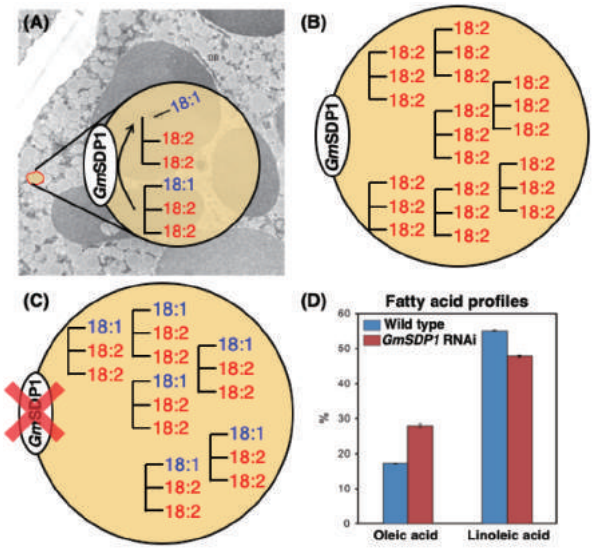

Figure 2. Quality and quantity control of storage oils in oil bodies by GmSDP1 in soybean (*Glycine max* L.). (A) Storage oils are accumulated as triacylglycerol (TAG) in oil bodies. Four Glycine max SUGAR DEPENDENT-1s (GmSDP1), which are types of lipase, have an activity for releasing fatty acids from TAG. GmSDP1s degrade oleic acid (18:1), so that the content of linoleic acid (18:2) in seeds increases (B), whereas the content of oleic acid rises in GmSDP1-knockdown plants compared to the wild-type plants due to reductions in the degradation of oleic acid (C, D).

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 to improve 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*. 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 sections: 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 stage.
- 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.

Publication List:

〔Original papers〕

- Goto-Yamada, S., Oikawa, K., Bizan, J., Shigenobu, S., Yamaguchi, K., Mano, S., Hayashi, M., Ueda, H., Hara-Nishimura, I., Nishimura, M., and Yamada, K. (2019). Sucrose starvation induces microautophagy in plant root cells. Front. Plant Sci. *10*, 1604. doi: 10.3389/fpls.2019.01604
- Kanai, M., Yamada, T., Hayashi, M., Mano, S., and Nishimura, M. (2019). Soybean (*Glycine max* L.) triacylglycerol lipase GmSDP1 regulates the quality and quantity of seed oil. Sci. Rep. *9*, 8924. doi: 10.1038/s41598-019-45331-8
- Kozuka, T., Sawada, Y., Imai, H., Kanai, M., Hirai, M.Y., Mano, S., Uemura, M., Nishimura, M., Kusaba, M., and Nagatani, A. (2020). Regulation of sugar and storage oil metabolism by phytochrome during de-etiolation. Plant Physiol. *182*, 1114-1129. doi: 10.1104/pp.19.00535
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- Yamauchi, S., Mano, S., Oikawa, K., Hikino, K., Teshima, K.M., Kimori, Y., Nishimura, M., Shimazaki, K.I., and Takemiya, A. (2019). Autophagy controls reactive oxygen species homeostasis in guard cells that is essential for stomatal opening. Proc. Natl. Acad. Sci. USA *116*, 19187-19192. doi: 10.1073/pnas.1910886116

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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 over 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 the 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 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, 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.

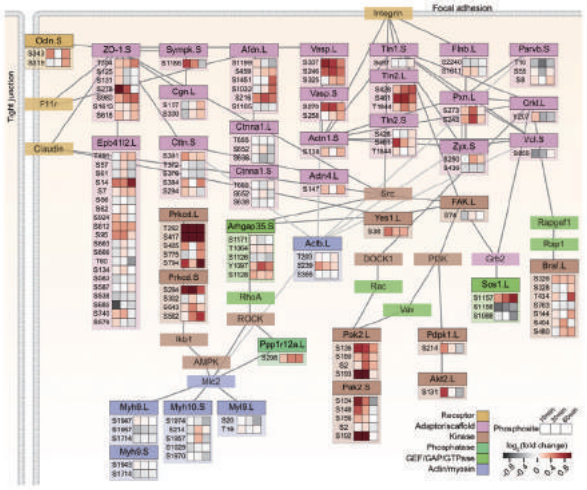

Figure 1. Phosphorylation changes of tight junction and focal adhesion components upon force stimulation in *Xenopus laevis* embryos.

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

The kinase substrate enrichment analysis based on the 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 activated Erk2 regulates integrity and stiffness of the embryonic epithelial cells. The centrifugal force to embryos accumulates F-actin, the tight junctional protein ZO-1 and the cell adhesion molecule cadherin 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.

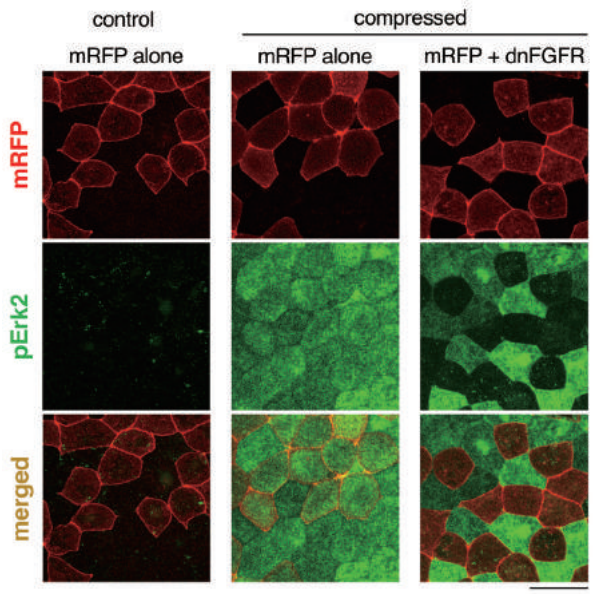

Figure 2. ERK2 is activated by a mechanical force (compression) in *Xenopus* embryonic cells in an FGFR-dependent manner.
Membrane RFP and dominant negative FGFR (dnFGFR) were expressed and compressed for 5 minutes at the gastrula stage. Embryos were stained with anti phoshorylated-ERK2 antibody (green) and the 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 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 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 stiffening 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 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 its enhancer activity in the notochord-specific expression is 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*. These results along with other data predicted an EvoDevo scenario in which *BfBra2* is more ancestral and mother-like, while *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.

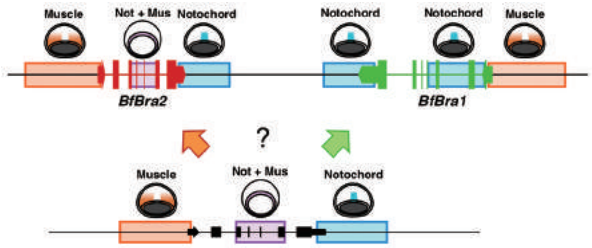

Figure 3. A summary of results of the present reporter assay and their possible interpretation.
Enhancer activity of 5' upstream sequences in muscle is shown in brown and that of the 3' downstream in notochord is in blue. The activity *BfBra2* introns in muscle and notochord is shown in purple.

V. Light-dependent swimming behavior of coral larvae in *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. 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 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.

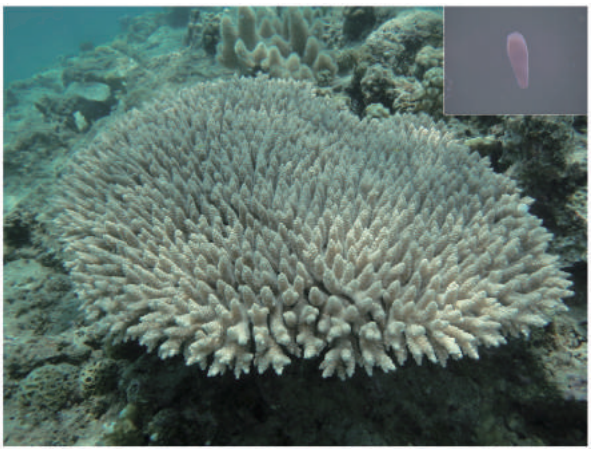

Figure 4. A wild colony of *Acropora tenuis* (photo taken by Masayuki Hatta at Ochanomizu University). The inset shows a planula larvae of *A. tenuis*.

Publication List:

〔Original papers〕

- Hashimoto, Y., Kinoshita, N., Greco, T.M., Federspiel, J.D., Jean Beltran, P.M., Ueno, N., and Cristea, I.M. (2019). Mechanical force induces phosphorylation-mediated signaling that underlies tissue response and robustness in *Xenopus* embryos. Cell Syst. *8*, 226-241. doi: 10.1016/j.cels.2019.01.006
- Ishii, Y., Maruyama, S., Takahashi, H., Aihara, Y., Yamaguchi, T., Yamaguchi, K., Shigenobu, S., Kawata, M., Ueno, N., and Minagawa, J. (2019). Global shifts in gene expression profiles accompanied with environmental changes in cnidarian-dinoflagellate endosymbiosis. G3-Genes Genom. Genet. *9*, 2337-2347. doi: 10.1534/g3.118.201012
- Itoh, M., Dai, H., Horike, S.-I., Gonzalez, J., Kitami, Y., Meguro-Horike, M., Kuki, I., Shimakawa, S., Yoshinaga, H., Ota, Y., Okazaki, T., Maegaki, Y., Nabatame, S., Okazaki, S., Kawawaki, H., Ueno, N., Goto, Y.-I., and Kato, Y. (2019). Biallelic KARS pathogenic variants cause an early-onset progressive leukodystrophy. Brain *142*, 560-573. doi: 10.1093/brain/awz001
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- Sakai, Y., Hatta, M., Furukawa, S., Kawata, M., Ueno, N., and Maruyama, S. (2020). Environmental factors explain spawning day deviation from full moon in the scleractinian coral *Acropora*. Biol. Lett. *16*, 20190760. doi: 10.1098/rsbl.2019.0760
- Virgirinia, R.P., Jahan, N., Okada, M., Takebayashi-Suzuki, K., Yoshida, H., Nakamura, M., Akao, H., Yoshimoto, Y., Fatchiyah, F., Ueno, N., and Suzuki, A. (2019). Cdc2-like kinase 2 (Clk2) promotes early neural development in *Xenopus* embryos. Dev. Growth Diff. *61*, 365-377. doi: 10.1111/dgd.12619

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 morphogenesis have been identified, but it is still uncertain how either spatial and temporal information are established and converted to morphology during embryogenesis. Therefore, our aim is to understand the mechanism by which this spatial information is established and how temporal, or periodical, information is converted into morphology through the application of several different approaches.

Secreted signal molecules are important in forming spatial information during the development of many tissues. These molecules are secreted from the cells that produce them and transported to surrounding cells, thus resulting in the formation of concentration gradients. Given that their concentration decreases in accordance with their distance from the source, their specific signal gradient 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 during tissue and embryo morphogenesis. However, in spite of the accumulation of genetic evidence, the molecular mechanism that regulates their distribution in certain developing tissues remains to be elucidated. To this end, we have visualized signal proteins and monitored their movement in tissues. In addition to this, we are currently examining the biochemical characteristics and functions of these molecules, which appear to affect how they are spread.

In contrast to secreted signal proteins, the segmental subregions of several specific tissues, like somites, appear not to be simply directed by the gradient of the signal molecules, but by a unique mechanism that functions periodically. Somites are sequentially generated in an anterior-to-posterior order via the conversion of 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 subdivision in the development of somites.

I. Effect of morphologic change in signal-producing cells during signal transmission in tissues

During morphogenesis, cells dynamically change their shape. Marked changes in the morphology of signal producing cells during tissue morphogenesis presumably result in the activation of signaling in neighboring cells. In early development of the vertebrate spinal cord, the most dorsal region, known as the roof plate, functions as an organizing center by secreting BMP and Wnt. These signaling proteins' concentration gradients generate interneuron subtype patterns in the dorsal spinal cord. Interestingly, as this patterning event nears completion, the spinal cord's morphology changes dynamically. Essentially, the neural tube lumen gradually shrinks, and a median septum forms along the dorsoventral axis. A recent study in zebrafish embryos revealed that the roof plate stretches during this morphologic change. This finding suggests that the processes of these stretched roof-plate cells are involved in intercellular communication and that the stretching of roof-plate cells induces changes in cells that respond to signals from the roof plate.

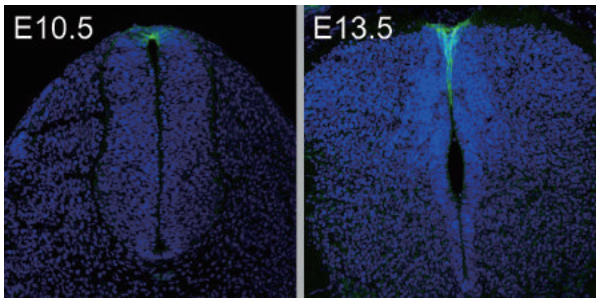

Figure 1. Stretching of Wnt expressing cells during spinal cord development in the mouse. Roof plate cells act as an organizing center in early development of spinal cord (embryonic day 10.5; E10.5). Then, these cells are stretched along the dorso-ventral axis (E13.5) and provide Wnt ligands to neural progenitor cells close to the tip of extended roof plate cells.

In adult mice, neural progenitor/stem cells localize around the shrunken lumen of the spinal cord, and this area is referred to as the central canal. The central canal is connected to the ventricle, where neural progenitor/stem cells localize in the forebrain. As the proliferation of neural progenitor/stem cells in the brain are regulated by secreted signaling molecules, including Wnt, it is plausible that spinal cord progenitor/stem cells are regulated in a similar manner. In the developing spinal cord, *Wnt1* and *Wnt3a* are specifically expressed in the roof plate and perform numerous roles, including regulating the proliferation and specification of dorsal interneurons prior to the mid-gestation stage. However, genetic studies of *Wnt1*- and *Wnt3a*-deficient mutant mouse embryos yielded no information regarding the roles of the respective proteins in later stages, as neither *Wnt1*- nor *Wnt3a*-deficient single mutants exhibit a roof plate–related phenotype, and most of the double mutants die before embryonic day 12.5 (E12.5).

Recently, we found that the expression of Wnt1 and Wnt3a is maintained in stretched roof-plate cells (Figure 1). Roof plate-specific cKO for Wls, which is essential for Wnt secretion, revealed that Wnt secretion from roof-plate cells was required for their coordinated elongation. Furthermore,

proliferation was reduced in ependymal cells in Wls cKO embryos during the development and regeneration of the spinal cord (Figure 2). Thus, dynamic change in cell extension can create novel roles for signal-producing cells.

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

To examine the mobility of Wnt3a in an extracellular situation, 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 because of its likely interaction with extracellular matrices. Further precise analyses revealed that HMW complexes were less mobile than relatively smaller ones.

We showed that the trimer and larger HMW complexes of Wnt3a proteins could be dissociated via 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 the assembly and dissociation of dissociable oligomers modulate Wnt signaling range (Figure 3).

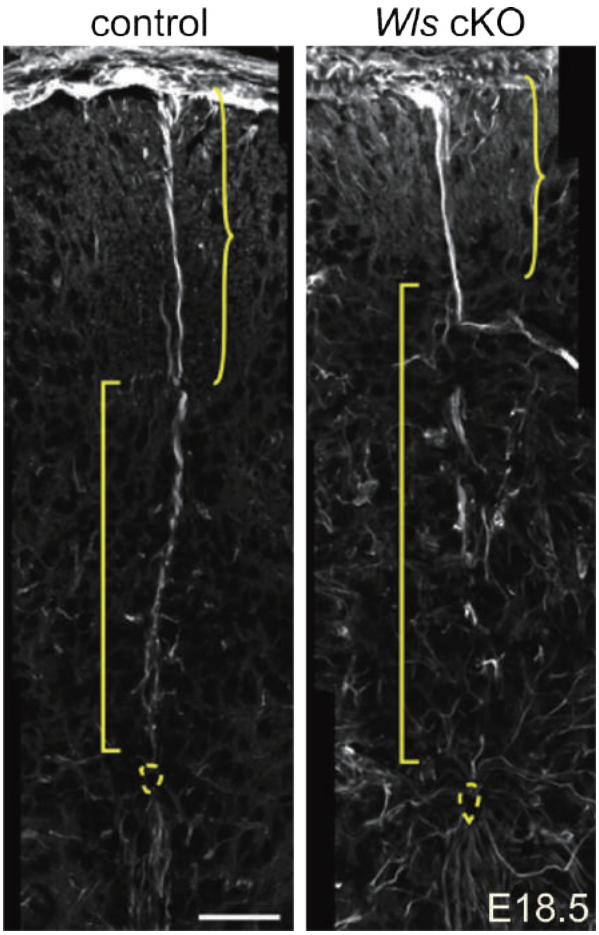

Figure 2. Impaired morphology of stretched roof plate cells in Wnt-defective embryos (Wls cKO). Extensions are indicated by brackets.

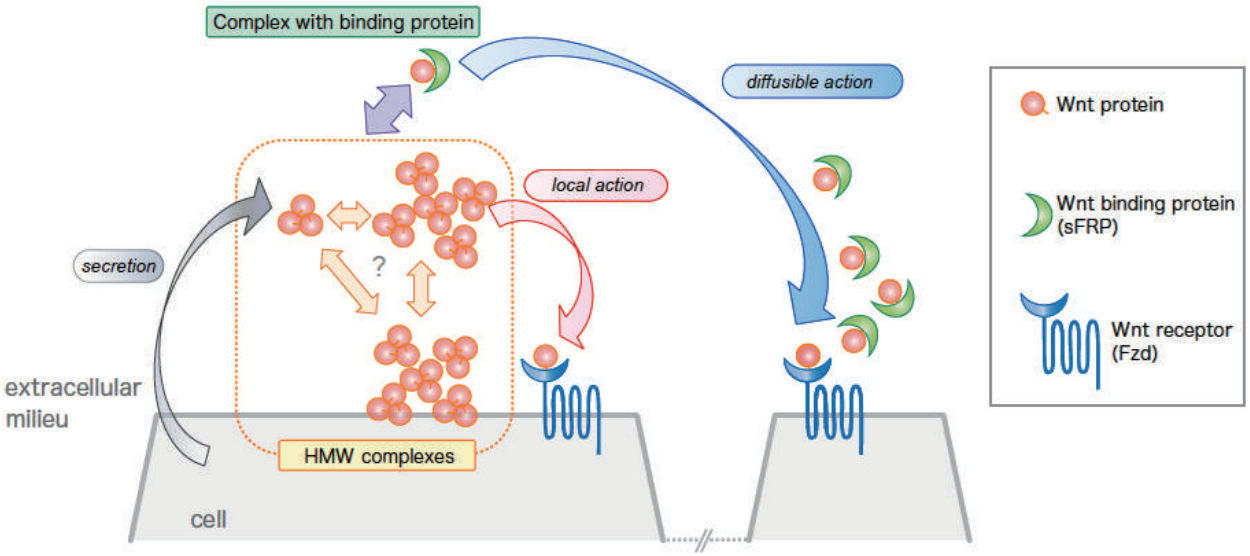

Figure 3. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu although it is uncertain when the assembly to the HMW complex occurs during the process of Wnt secretion. The HMW complex is 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. Due to this dissociation, Wnt turns to be more mobile and its diffusion range is expanded (diffusible action).

III. Molecular mechanism of somite development

Somites are segmental epithelial blocks located symmetrically on either side of the neural tube. Somitogenesis periodically proceeds 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 within 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 results 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 intra-cellular molecules, as well as FGF and Wnt signaling, are involved in this conversion. It has been generally considered that Mesp of the bHLH transcriptional regulator plays a critical role in this conversion. In mice, the prospective segmentation boundary is periodically established in the anterior PSM at the rostral border of the *Mesp2* expression domain. Conversely, recent studies of 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 mice and zebrafish. *Ripply* genes encode ~100 amino acid proteins, that degrades Tbx6 proteins, which is involved in the positioning of the segmentation boundary. We have examined the mechanism of this conversion by focusing on the interaction between Ripply and other molecules involved in this conversion, including Hairy and FGF pathway molecules. Currently, we are visualizing the spatio-temporal shifts of these molecules in the PSM in wild-type and segmentation-defective zebrafish embryos.

Publication List:

〔**Original papers**〕

- Ban, H., Yokota, D., Otosaka, S., Kikuchi, M., Kinoshita, H., Fujino, Y., Yabe, T., Ovara, H., Izuka, A., Akama, K., Yamasu, K., Takada, S., and Kawamura, A. (2019). Transcriptional autoregulation of zebrafish *tbx6* is required for somite segmentation. Development *146*, dev177063. doi: 10.1242/dev.177063
- Kikuchi, M., Nishimura, T., Saito, D., Shigenobu, S., Takada, R., Gutierrez-Triana, J.A., Cerdán, J.L.M., Takada, S., Wittbrodt, J., Suyama, M., and Tanaka, M. (2019). Novel components of germline sex determination acting downstream of foxl3 in medaka. Dev. Biol. *445*, 80-89. doi: 10.1016/j.ydbio.2018.10.019
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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.

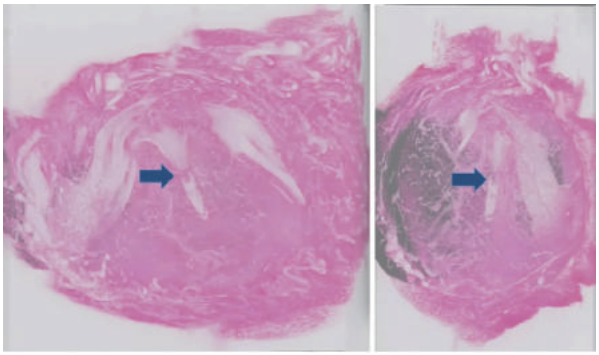

Figure 1. Three-dimensional image reconstruction of the pregnant uterus. A uterus obtained 5 days after fertilization was sectioned and images were digitized by an image scanner, and reconstructed three-dimensionally to observe the relationship between the embryo and the maternal tissue. The right panel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

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.

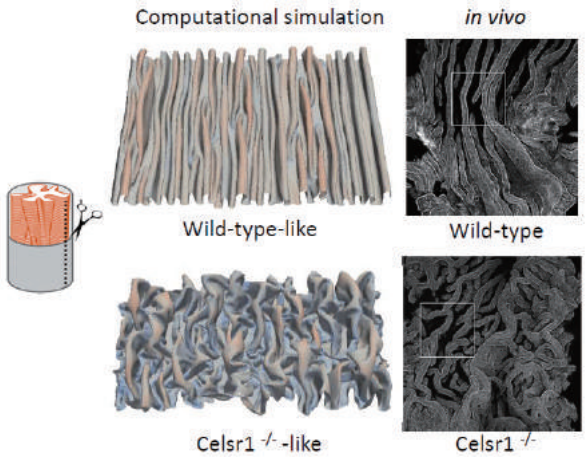

Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

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.

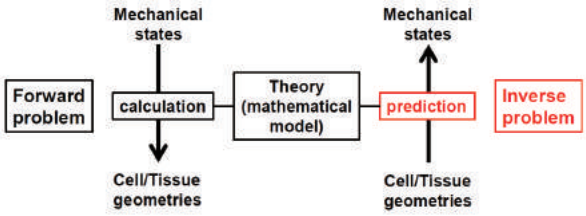

Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

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

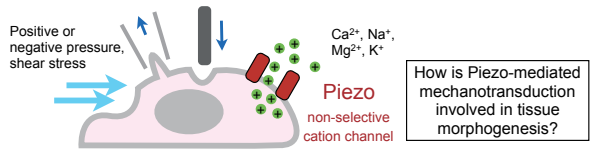

Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

main mechanosensor in sensory neurons for light touch sensation, 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.

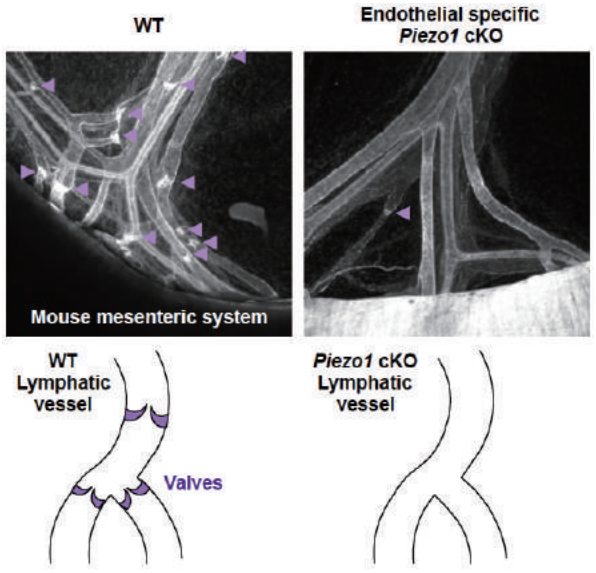

Figure 5. Reduced number of lymphatic valves in mice lacking PIEZO1 in endothelial cells.

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〕

- Đặng, T.C., Ishii, Y., Nguyen, V.D., Yamamoto, S., Hamashima, T., Okuno, N., Nguyen, Q.L., Sang, Y., Ohkawa, N., Saitoh, Y., Shehata, M., Takakura, N., Fujimori, T., Inokuchi, K., Mori, H., Andrae, J., Betsholtz, C., and Sasahara, M. (2019). Powerful homeostatic control of oligodendroglial lineage by PDGFRα in adult brain. Cell Rep. *27*, 1073-1089. doi: 10.1016/j.celrep.2019.03.084
- Honda, H., Abe, T., and Fujimori, T. (2020). The chiral looping of the embryonic heart is formed by the combination of three axial asymmetries. Biophys. J. *118*, 742-752. doi: 10.1016/j.bpj.2019.11.3397
- Kamemizu, C., and Fujimori, T. (2019). Distinct dormancy progression depending on embryonic regions during mouse embryonic diapause. Biol. Reprod. *100*, 1204-1214. doi: 10.1093/biolre/ioz017
- Kawasoe, R., Shinoda, T., Hattori, Y., Nakagawa, M., Pham, T.Q., Tanaka, Y., Sagou, K., Saito, K., Katsuki, S., Kotani, T., Sano, A., Fujimori, T., and Miyata, T. (2020). Two-photon microscopic observation of cell-production dynamics in the developing mammalian neocortex in utero. Dev. Growth Differ. *62*, 118-128. doi: 10.1111/dgd.12648
- Kiyonari, H., Kaneko, M., Abe, T., Shioi, G., Aizawa, S., Furuta, Y., and Fujimori, T. (2019). Dynamic organelle localization and cytoskeletal reorganization during preimplantation mouse embryo development revealed by live imaging of genetically encoded fluorescent fusion proteins. Genesis *57*, e23277. doi: 10.1002/dvg.23277
- Sone, E., Noshiro, D., Ikebuchi, Y., Nakagawa, M., Khan, M., Tamura, Y., Ikeda, M., Oki, M., Murali, R., Fujimori, T., Yoda, T., Honma, M., Suzuki, H., Ando, T., and Aoki, K. (2019). The induction of RANKL molecule clustering could stimulate early osteoblast differentiation. Biochem. Biophys. Res. Commun. *509*, 435-440. doi: 10.1016/j.bbrc.2018.12.093
- Yamada, K., Hamashima, T., Ishii, Y., Yamamoto, S., Okuno, N., Yoshida, N., Yamada, M., Huang, TT., Shioda, N., Tomihara, K., Fujimori, T., Mori, H., Fukunaga, K., Noguchi, M., and Sasahara, M. (2018). Different PDGF receptor dimers drive distinct migration modes of the mouse skin fibroblast. Cell Physiol. Biochem. *51*, 1461-1479. doi: 10.1159/000495594

〔Review article〕

- Koyama, H., Shi, D., and Fujimori, T. (2019). Biophysics in oviduct: Planar cell polarity, cilia, epithelial fold and tube morphogenesis, egg dynamics. Biophys. Physicobiol. *16*, 89-107. doi: 10.2142/biophysico.16.0_89

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

We have 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 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 endo-

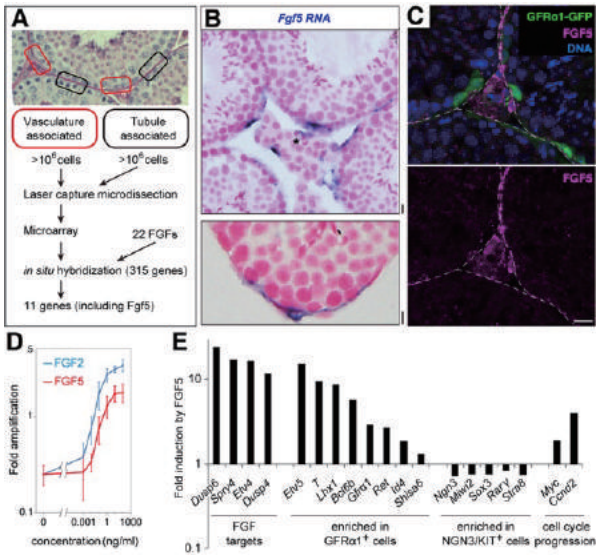

Figure 1. (A) Outline of the screening for genes preferentially expressed in the vasculature-associated region. (B) Representative ISH images for *Fgf5* (blue) in testis sections, counterstained with nuclear fast red.. (C) Representative image of an inter-tubular region of a GFRα1-GFP mouse testis stained 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).

thelial 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).

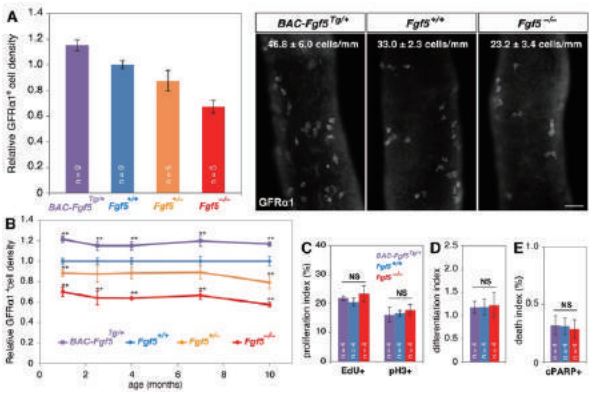

Figure 2. (A) Average densities ± SEM of GFRα1⁺ cells and representative IF images of whole-mount seminiferous tubules for GFRα1 (right) of 2.5 month-old mice with indicated genotypes. Scale bar, 50μm. (B) Average densities ± SEM of GFRα1⁺ cells in mice with indicated genotypes and ages. (C–E) Indexes 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.

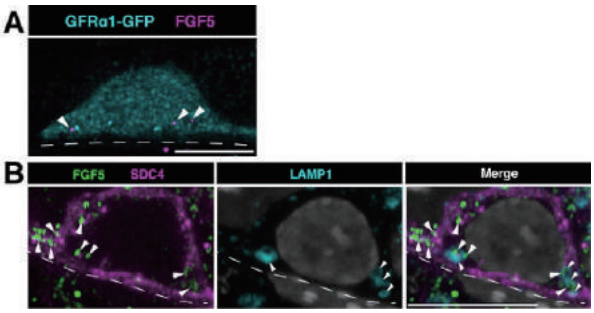

Figure 3. (A) Representative images of GFRα1-GFP⁺ (cyan) cells exhibiting the speckled cytoplasmic staining of FGF5 (magenta, white arrowheads). (B) Representative images of SDC4⁺ (magenta) cells co-stained for FGF5 (green) with LAMP1 (cyan). FGF5⁺ speckles (arrows) were often co-localized with LAMP1⁺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

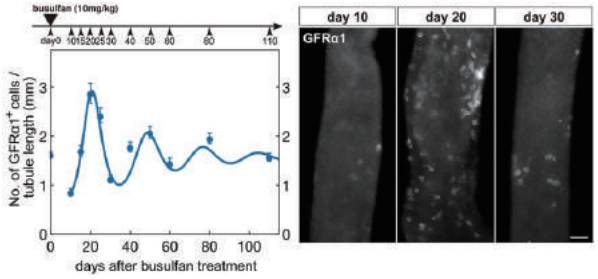

Figure 4. Observed kinetics of the GFRα1⁺ cell density following busulfan treatment in WT (left), and examples of IF images of whole-mount seminiferous tubules stained for GFRα1 (right). Model results (curves) compared to experimental measurements (dots) of the average GFRα1⁺ cell density following busulfan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

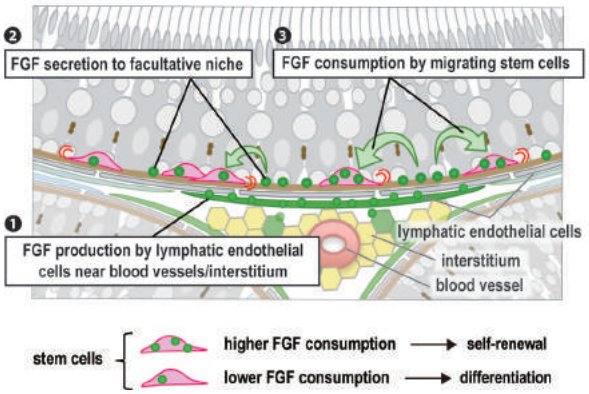

Figure 5. A conceptual diagram of mitogen competition leading to stem cell density homeostasis. Limited supply of FGF from a subset of lymphatic endothelial cells in proximity to vasculature plays a key role in stem cell density homeostasis.

Publication List:

〔Original Papers〕

- 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. (2019). Competition for mitogens regulates spermatogenic stem cell homeostasis in an open niche. Cell Stem Cell *24*, 79-92. doi: 10.1016/j.stem.2018.11.013
- Nakamura, S., Hira, S., Fujiwara, M., Miyagata, N., Tsuji, T., Kondo, A., Kimura, H., Shinozuka, Y., Hayashi, M., Kobayashi, S., and Mukai, M. (2019). A truncated form of a transcription factor Mamo activates *vasa* in *Drosophila* embryos. Commun. Biol. *2*, 422. doi: 10.1038/s42003-019-0663-4
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- Yoshida, S. (2020). Mouse spermatogenesis reflects the unity and diversity of tissue stem cell niche systems. Cold Spring Harbor Perspectives in Biology, a036186. doi: 10.1101/cshperspect.a036186

Laboratory of Regeneration Biology

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Comparative Regenerative Biology

We use animals with high regenerative abilities, such as planarians and newts, to understand the principle of regeneration. In particular, we investigate the difference between regenerative and non-regenerative animals to evoke said abilities from non-regenerative animals. We have already succeeded in achieving this with planarians, which were able to regenerate their heads through RNAi (Umesono *et al*., 2013 Nature) in addition to accomplishing functional joint regeneration in frogs through the activation of reintegration systems (Tsutsumi *et al*., 2016 Regeneration).

We are trying to induce limb-regeneration ability in frogs, as they lose the capability to achieve complete limb regeneration after metamorphosis. Thus, we are focusing on the *Sonic hedgehog* (*Shh*) enhancer MFCS1 (mammals-fishes conserved sequence 1), since it was suggested that the loss of MFCS1 activity after metamorphosis might cause a failure to achieve the aforementioned limb regeneration in adult frogs (Yakushiji *et al*., 2009). When we compared the MFCS1 sequences between newts (*C.p*. and *P.w*.) and frogs (*X.l*. and *X.t*.), newts were found to possess several specific sequences (Figure 1). Thus, we subsequently planned to swap the MFCS1 sequences between newts and frogs using CRISPR/Cas9 technology.

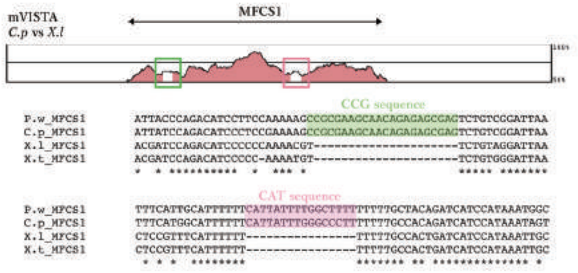

Figure 1. Comparison of the MFCS1 sequences between newts and frogs.

We then prepared various guide RNAs to swap these sequences, and succeeded in the production of mosaically targeted Iberian ribbed newts (*Pleurodeles waltl: P.w*., Figure 2) and frogs (*Xenopus tropicalis: X.t*.). Thus, we have tried the PITch method to knock-in the newt-MFCS1 fragment into the frog-MFCS1 site, but have yet to succeed in achieving this.

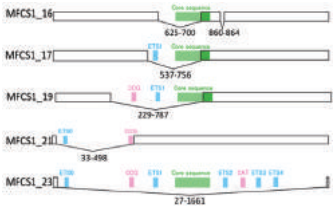

Figure 2. Deletion patterns of the newt-MFCS1 region (ca. 1.6 kb) obtained by microinjection of several different cocktails of gRNAs with CRISPR/Cas9.

Trial for the cultivation of planarian embryonic and adult pluripotent stem cells

We developed an isolation method of adult pluripotent stem cells (aPSC) from planarian using FACS and tried to cultivate them under in vitro culture conditions, but we have yet to succeed in propagating these cells. LCDM and EPSCM media have recently been developed to cultivate mouse EPS (Expanded Potential Stem) cells, which can differentiate both embryonic and extra-embryonic cells. Thus, it is expected that these media might work to cultivate embryonic pluripotent stem cells (ePSC) derived from non-mammalian species. Thus, we tried to cultivate planarian embryonic cells by LCDM and EPSCM media and got healthy planarian ePSC like-aggregates in vitro (Figure 3). We also attained similar healthy cell-aggregates from planarian aPSC after isolation using FACS. However, we unfortunately could not detect the active proliferative activity of these cells in these culture media so far.

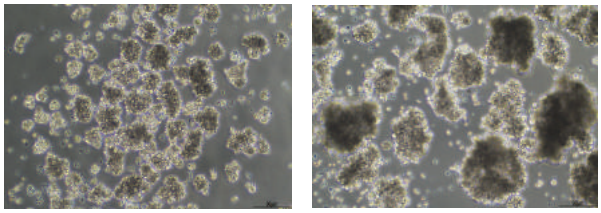

Figure 3. planarian ePSC-aggregates formed in EPSC (left) and EPSCM (right) media.

Publication List:

〔Original Papers〕

- Kishimoto, K., Sugano-Yasunaga, W., Taniguchi, A., Agata, K., Nonaka, S., and Funayama, N. (2019). Skeleton construction upon local regression of the sponge body. Dev Growth Differ *61*, 485-500. doi:10.1111/dgd.12636
- Matsunami, M., Suzuki, M., Haramoto, Y., Fukui, A., Inoue, T., Yamaguchi, K., Uchiyama, I., Mori, K., Tashiro, K., Ito, Y., *et al*. (2019). A comprehensive reference transcriptome resource for the Iberian ribbed newt Pleurodeles waltl, an emerging model for developmental and regeneration biology. DNA Res *26*, 217-229. dio:10.1093/dnares/dsz003
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〔Review article〕

- Nakanoh, S., and Agata, K. (2019). Evolutionary view of pluripotency seen from early development of non-mammalian amniotes. Dev Biol *452*, 95-103. doi: 10.1016/j.ydbio.2019.04.014

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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 technique.

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).

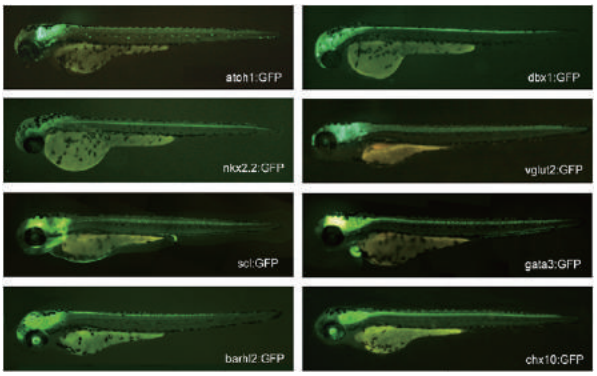

Figure 1. Examples of transgenic fish expressing GFP in specific classes of neurons.

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.

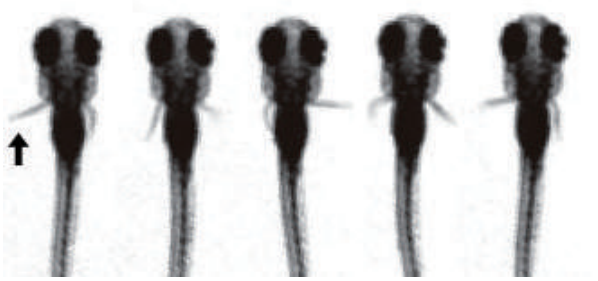

Figure 2. Rhythmic movements of the pectoral fin (arrow) during swimming in larval zebrafish.

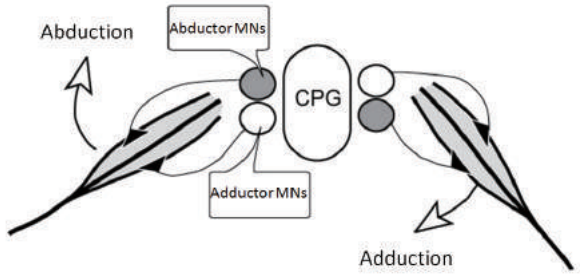

Figure 3. Schematic of rhythmic movements of pectoral fins during swimming. CPG, Central Pattern Generator.

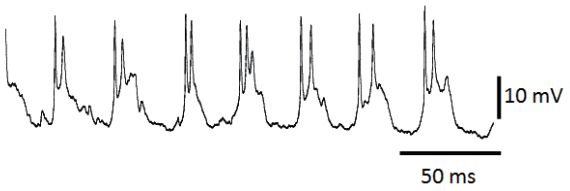

Figure 4. Rhythmic firings of an abductor motoneuron during swimming.

III. Functional analysis of V1 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 (see Section IV). The role of the latter (ipsilaterally-projecting inhibitory neurons) is less clear.

V1 neurons are one class of neurons that are defined by the expression of a transcription factor En1 and constitute major components of ipsilaterally-projecting inhibitory neurons in the spinal cord. To investigate the function of V1 neurons, we genetically ablated spinal V1 neurons in larval zebrafish using diphtheria toxin A (En1-DTA fish, Figure 5). From the analysis of the En1-DTA fish, we revealed two roles of V1 neurons.

First, we found that the swimming frequency was declined in the En1-DTA fish (Figure 6). The results show that V1

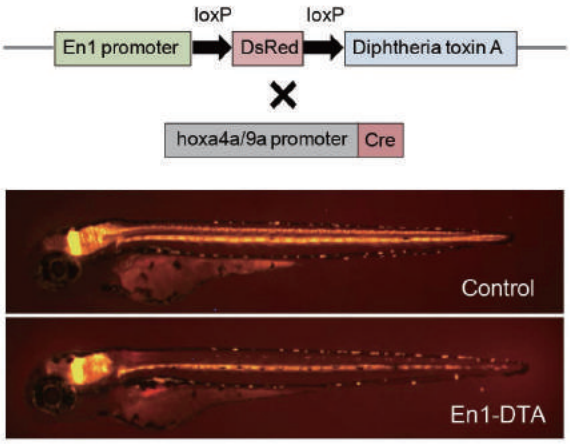

Figure 5. Ablation of spinal V1 neurons by using the Cre-loxP system. DTA, diphtheria-toxin A.

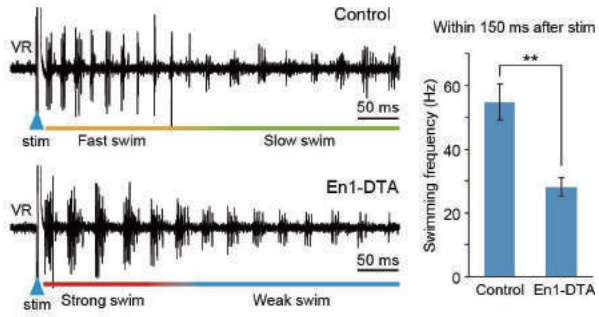

Figure 6. Ventral root recordings of fictive swimming elicited by electrical stimulations (stim) in a control and an En1-DTA fish (left). Swimming frequency of control and En1-DTA fish during the initial phase of swimming elicited by electrical stimulation (right).

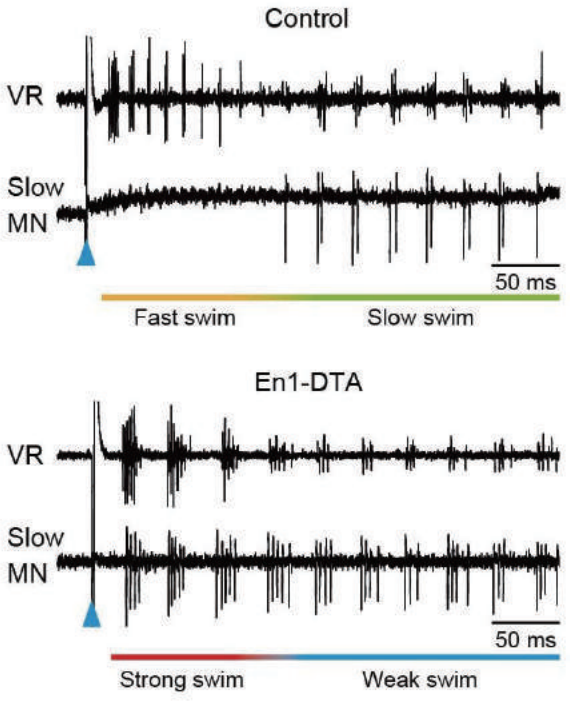

Figure 7. Activity of slow type motoneurons (MNs) in control and En1-DTA fish during fictive swimming.

neurons play an important role for controlling locomotion speed.

Second, we showed that V1 neurons play an important role in the selection of active sets of neurons. Slow-type motor neurons, which project slow muscle, are known to be inactive during strong/fast movements in larval zebrafish. In the En1-DTA fish, however, we found that slow-type motor neurons were vigorously active during strong movements (Figure 7). We investigated the mechanism underlying this phenotype. In the wild type, slow-type MNs were found to receive strong inhibitory inputs. In the En1-DTA fish, these strong inhibitory inputs were found to be absent. The results indicate that V1 neurons are the source of the strong inhibition onto slow-type MNs and that the inhibition play a crucial role in suppressing spiking activities of slow-type MNs during strong/fast swimming.

IV. Functional diversity of glycinergic commissural inhibitory neurons in larval zebrafish

Coordinated movements of the left and right side of the body is critical for any types of locomotion including walking, flying and swimming. The coordination is mainly mediated by commissural neurons located in the spinal cord. In fish swimming, it is known that commissural inhibitory neurons in the spinal cord ensure left-right alternating movements. The developmental origin of these commissural inhibitory neurons, however, has been elusive. We investigated anatomy and function of two commissural inhibitory neuron types, dI6*dmrt3a* and V0d, derived from the pd6 and p0 progenitor domains, respectively. We found that both of these commissural neuron types have monosynaptic, inhibitory connections to neuronal populations active during swimming, supported their role in providing inhibition to the contralateral side. V0d neurons tend to fire during faster and stronger movements, while dI6*dmrt3a* neurons tend to fire more consistently during swimming. Ablation of dI6*dmrt3a* neurons leads to an impairment of left-right alternating activity through abnormal co-activation of motor neurons on both sides of the spinal cord. Our results suggest that dI6*dmrt3a* and V0d commissural inhibitory neurons synergistically provide inhibition to the opposite side across different swimming behaviors (Figure 8).

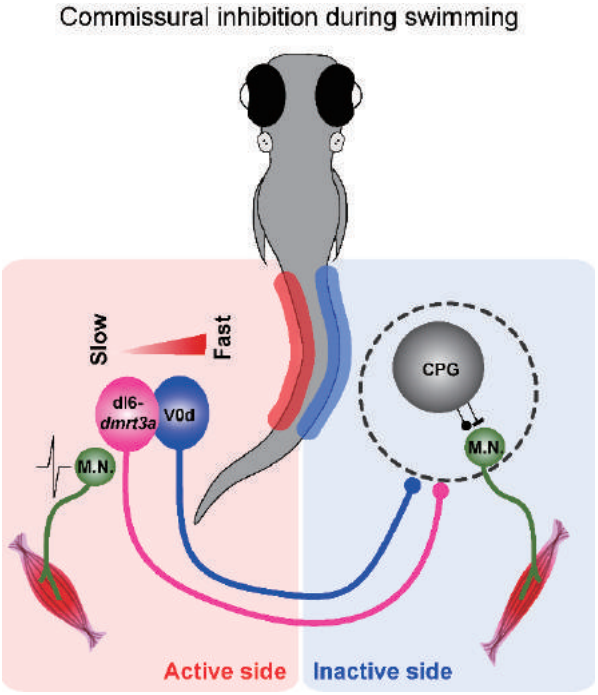

Figure 8. Summary of the proposed role of dI6*dmrt3a* and V0d neurons during swimming. CPG, central pattern generator

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LABORATORY OF NEUROPHYSIOLOGY

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We believe that the best way to understand mechanisms underlying a certain system is to reconstruct the system as it exists. By utilizing novel computer technologies, we are accordingly trying to build the systems based on psychophysical and ecological viewpoints in particular to understand systems of animal perception and behavior. This type of methodology based on computers will pave the way for biology in the future.

I. Psychophysical study of medaka fish

One of our major subjects is the psychophysical and computational study of medaka (*Oryzias latipes*, Matsunaga & Watanabe, 2010). We have recently made progress in studies of prey-predator interaction using both these organisms and zooplankton. Visual motion cues are one of the most important factors for eliciting animal behaviors, 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 we analyzed the predation behavior in response to computer-generated plankton. To achieve this, virtual plankton models were programmed on a computer and presented to predator medaka. As a result of this analysis, we confirmed that medaka exhibited predation behavior against several characteristic movements of the virtual plankton, particularly against a swimming pattern that could be characterized as a pink noise motion. The analysis of predator-prey interactions via pink noise motions will be a research field of great interest moving forward (Matsunaga & Watanabe, 2012).

In recent years, we have made progress in the study of 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 utilizing biological motion stimuli, which in this case was the depiction of a moving creature by using just a few isolated points, we examined whether physical motion information is involved in the induction of shoaling behavior. We consequently found that the presentation of virtual biological motion can clearly induce shoaling behavior, and 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 further enhance our understanding of how non-human animals extract and process information which is vital for their survival (Nakayasu & Watanabe, 2014).

Additionally, 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 actual medaka (Figure 1). These virtual 3D models will allow us to represent medaka more faithfully as well as 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), and on underwater imaging technology (Abe *et al*., 2019 and Yamamoto *et al*., 2019).

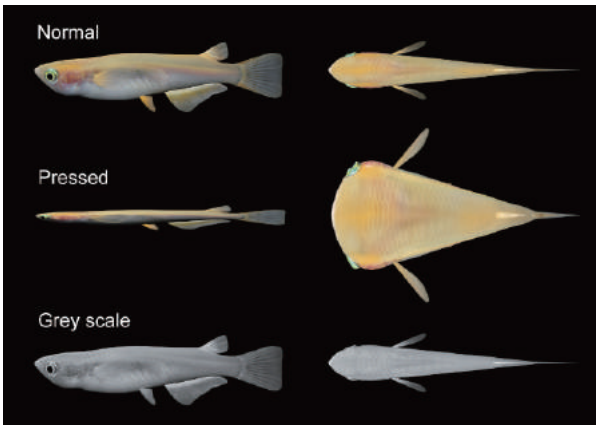

Figure 1. Virtual medaka fish.

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. One recent focus of this debate is the flash-lag effect, in which a moving object is perceived to head towards a flashed object when both objects are aligned in an 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

Figure 2. Monstre Benham Illusions. Benham Top is a black and white top that, when rotating, causes people to perceive illusory colors such as red or blue. Illusory colors were first discovered by the German physicist Gustav Fechner, and the Benham top was built by the English journalist Charles Benham. We have implemented a version of the Benham top that instead of spinning, works like a flip book (demo, https://www.sonycsl.co.jp/news/9164/).

novel visual illusions, such as the shelf-shadow illusion (3rd Award of the 5th Illusion Contest, https://doi.org/10.6084/m9.figshare.6137558) and the Monstre Benham illusion (2nd Award of the 11th Visual Illusion and Auditory Illusion Contest, https://doi.org/10.6084/m9.figshare.10046534, Figure 2) .

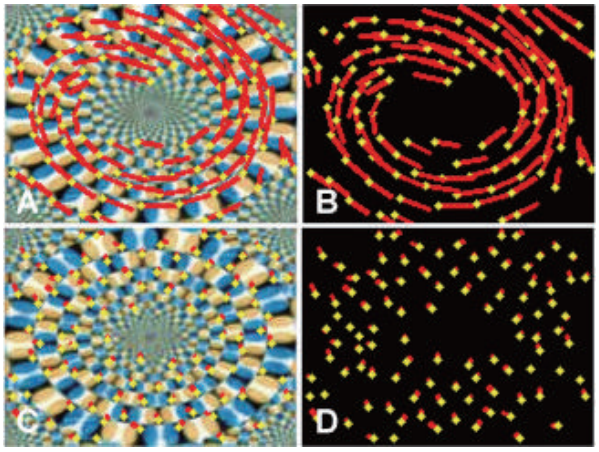

Figure 3. Optical flow vectors detected in the illusion. Red bars denote the direction and magnitude of vectors, yellow dots denote the start points of the vectors. A and B: Illusion, C and D: Non-Illusion.

In 2018, we successfully generated deep neural networks (DNNs) that represent the perceived rotational motion for illusion images that were not physically moving, yet similar to what we experience in human visual perception. (Figure 3, Watanabe *et al*., 2018). These DNN computer models will help to facilitate our future work on perception science. This experimental system was applied to studies on evolutionary illusion generator in collaborating with Dr. Lana Sinapayen (Sony CSL). (EIGen, https://github.com/LanaSina/evolutionary_illusion_generator. Sinapayen and Watanabe, 2020).

III. Ecological study of tactics in predators and prey.

We are interested in the behavioral interactions between predators and prey not only concerning the psychophysical aspect but also the ecological aspect. Predators and prey have developed various tactics to overcome each other, in the process of the co-evolution. To elucidate the sophistication of these tactics, we have conducted experiments to examine the mechanisms and efficacies of predatory and antipredator behaviors of various animals such as snakes, frogs, fish, dragonflies and bats. During this fiscal year, we published a paper, which outlined the vulnerability of preemptive actions of both predator snakes and prey frogs, and the possibility that this vulnerability instigates a 'waiting game' between these animals (Nishiumi & Mori, 2020). Several on-going studies were also presented at conferences, which were about the tactics of predatory bats regarding echolocation. In addition, we have developed several experimental technologies for those studies, which present interactive virtual predators to prey animals based on animation or robotics (Figure 4). These technologies do not require specific uncommon hardware or software as previous technologies have required (*i.e*. The present technologies work in Windows and Mac OS with low-price webcams and several free computer libraries), and thus, are expected to contribute wider range of animal research.

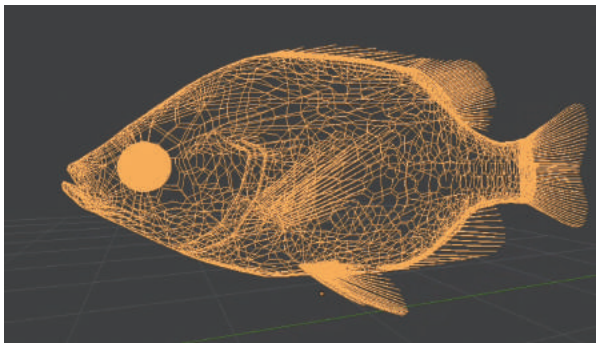

Figure 4. A computer animation of the virtual predatory fish.

Publication List:

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- Abe, E., Yasugi, M., Takeuchi, H., Watanabe, E., Kamei, Y., and Yamamoto, H. (2019). Development of omnidirectional aerial display with aerial imaging by retro-reflection (AIRR) for behavioral biology experiments. Opt. Rev. *26*, 221-229. doi: 10.1007/s10043-019-00502-w
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- Yamamoto, H., Abe, E., Yasugi, M., Watanabe, E., and Takeuchi, H. (2019). Aquatic information display and its applications for behavioral biology experiments. Proc. SPIE 10997, Three-Dimensional Imaging, Visualization, and Display 2019, 1099707. doi: 10.1117/12.2521225

DIVISION OF EVOLUTIONARY BIOLOGY

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	WEN, Hsin-I
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	OGAWA, Yuko
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	HASEBE, Yuki

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*.

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 that a transporter mutant, which belongs to the *ABC* gene family, exhibited a cell shape abnormality. 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 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, including histone 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, in land plants, stem cells are formed from differentiated cells during post-embryonic development and regeneration, indicating that land plants have an intrinsic ability to regulate epigenetic memory to initiate a new gene regulatory network. However, it is less well understood how epigenetic modifications are locally regulated to influence specific genes necessary for cellular changes without affecting other genes in a genome. In this study, we found that ectopic induction of the AP2/ERF transcription factor STEMIN1 in leaf cells of the moss *Physcomitrella patens* decreases a repressive chromatin mark, histone H3 lysine 27 trimethylation (H3K27me3), on its direct target genes before cell division, resulting in the conversion of leaf cells to chloronema apical stem cells. STEMIN1 and its homologs positively regulate the formation of secondary chloronema apical stem cells from chloronema cells during development. Our results suggest that STEMIN1 functions in an intrinsic mechanism underlying local H3K27me3 reprogramming to initiate stem cell formation. Masaki Ishikawa is this study's coordinating researcher.

We also found that a component of the DNA repair machinery functions in the STEMIN1-induced stem cell formation in leaves. This study is mainly being conducted by Ruan de Villiers.

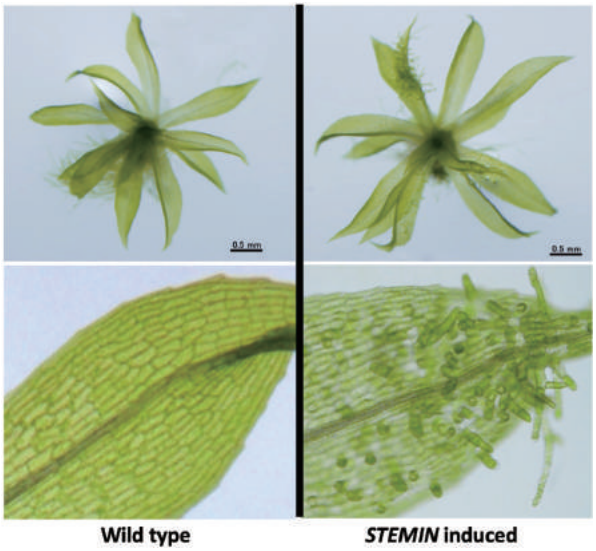

Figure 1. STEMIN can change leaf cells into stem cells.

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*.

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.

VIII. Establishment of a new single cell transcriptome method

Next-generation sequencing technologies have made it possible to carry out transcriptome analysis at the single-cell level. Single-cell RNA-sequencing (scRNA-seq) data provide insights into cellular dynamics, including intercellular heterogeneity as well as inter- and intra-cellular fluctuations in gene expression that cannot be studied using populations of cells. The utilization of scRNA-seq is, however,

restricted to cell types that can be isolated from their original tissues, and it can be difficult to obtain precise positional information for these cells in situ. Here, we established single cell-digital gene expression (1cell-DGE), a method of scRNA-seq that uses micromanipulation to extract the contents of individual living cells in intact tissue while recording their positional information. With 1cell-DGE, we could detect differentially expressed genes (DEGs) during the reprogramming of leaf cells of the moss Physcomitrella patens, identifying 6382 DEGs between cells at 0 and 24 h after excision. Furthermore, we identified a subpopulation of reprogramming cells based on their pseudotimes, which were calculated using transcriptome profiles at 24 h. 1cell- DGE with microcapillary manipulation can be used to analyze the gene expression of individual cells without detaching them from their tightly associated tissues, enabling us to retain positional information and investigate cell–cell interactions.

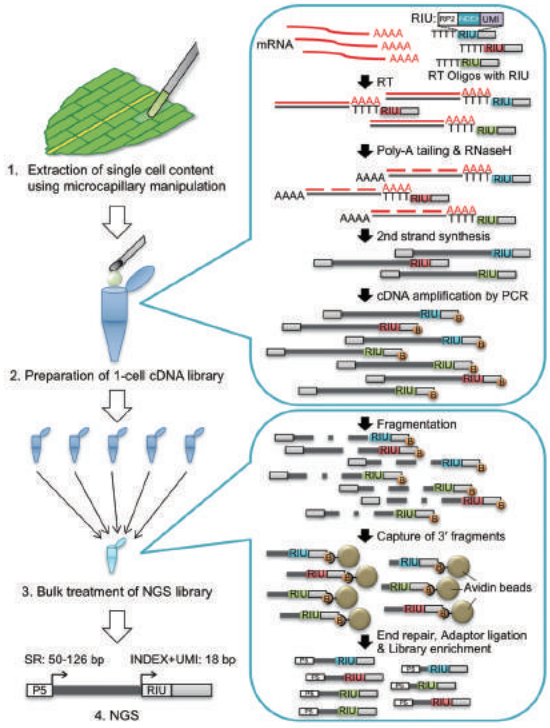

Figure 2. Schematic representation of the workflow of single cell – digital gene expression (DGE)

IX. 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.

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- Cai, H., Li, Q., Fang, X., Li, J., Curtis, N.E., Altenburger, A., Shibata, T., Feng, M., Maeda, T., Schwartz, J.A., Shigenobu, S., Lundholm, N., Nishiyama, T., Yang, H., Hasebe, M., Li, S., Pierce, S.K., and Wang, J. (2019). Data descriptor: A draft genome assembly of the solar-powered sea slug *Elysia chlorotica*. Sci. Data *6*, 190022. doi: 10.1038/sdata.2019.22
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〔Review article〕

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Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on Earth. In this type of 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 elucidate the molecular mechanisms of both symbiotic systems.

I. Root nodule symbiosis

I-1 Recruitment of a factor involved in lateral root development intro root nodule organogenesis

Plants develop lateral roots to efficiently absorb water and nutrients from soil. Additionally, legumes have acquired the ability to produce root nodules. We have demonstrated that a transcription factor involved in the initial stages of lateral root development regulates nodule development in coordination with NF-Y CAATA-box binding protein complex in *L. japonicus* (Figure 1A).

This factor is known as ASL18/LBD16. *L. japonicus ASL18/LBD16* is expressed in the nodule primordia as well as in the lateral root primordia. The former originates in the root cortex, and the latter develops from the pericycle. ASL18/LBD16 interacts with NF-Y subunits in vitro and in planta. The *ASL18/LBD16* expression domain overlaps those of *NF-Y subunit* genes in nodule primordia. *ASL18/LBD16* indicated genetic interaction with *NF-Y subunit* genes in nodule primordia development (Figure 1B), whereas *nf-y* subunit mutations did not affect the fewer lateral root phenotype associated with *asl18* mutants. Co-overexpression of *ASL18/LBD16* in concert with *NF-Y subunit* genes partially suppressed the nodule-defective phenotype of *daphne* mutants, where *NIN* expression in the root cortex was repressed.

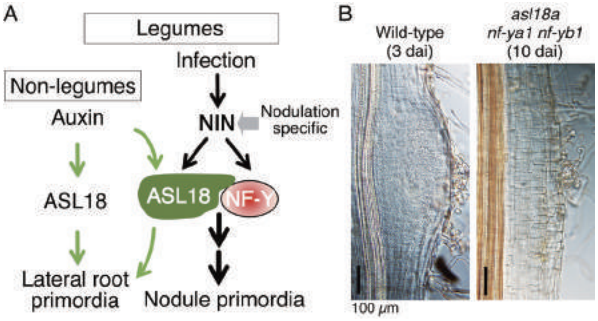

Figure 1. Recruitment of ASL18 into nodule development. (A) A model of ASL18-mediated pathway. (B) Nodule primordia in wild-type [3 days after inoculation (dai)] and *asl18a nf-ya1 nf-yb1* triple mutant roots (10 dai).

NIN is a nodulation-specific transcription factor initiating nodule primordia. This factor binds with the intronic *cis*-element in *ASL18/LBD16*. The binding sites and its flanking regions are highly conserved among legume *ASL18/LBD16* genes. The acquisition of this site in an ancestor of legume lineage could have influenced the *ASL18/LBD16* function in extant legumes. The interaction with NF-Y might confer the new function upon legume ASL18/LBD16 orthologs.

I-2 Host symbiotic transcription factor network regulates robust infection processes

Bacterial entry into root tissues is an essential process for establishing the symbiotic relationship between rhizobia and host legumes. Rhizobia are entrapped by curled root hairs and penetrate into the inner tissues of roots through infection threads (ITs), host-membranous and tubular paths, developed from infection foci. Multiple host transcription factors are involved in these processes.

CYCLOPS is a transcription factor commonly required for early signaling in both root nodule and AM symbioses and is essential for IT development. This factor directly regulates two transcription factor cording genes, *NIN* and *ERN1* (Figure 2A). Our analyses showed that *ERN1* also regulated *NIN* expression independently of CYCLOPS after inoculation. *NIN* expression was repressed more extensively in *cyclops ern1* than in each single mutant.

As was expected, ectopic expression of either *ERN1* or *NIN* suppressed the IT-defect of *cyclops* by the transcriptional hierarchy (Figure 2B). However, *CYCLOPS* and *NIN* did not recover ITs in *ern1*. Therefore, ERN1 has a function in common with CYCLOPS in *NIN* regulation, but it also has a different role from CYCLOPS and NIN. In keeping with this, *ern1* exhibited a unique symbiotic root hair phenotype. A depolarized balloon-shape root hair tip was observed in *ern1*, whereas *cyclops* and *nin* displayed excessive root hair curling, resulting in the rhizobia being entrapped (Figure 2C). The root hair response appeared to be arrested at an earlier stage of the deformation process in *ern1* than those observed in *cyclops* and *nin*.

These results highlight that transcription factors regulating symbiotic root hair responses constitute this complex network, and that they also interact with each other. This may reinforce the flow of the transcriptional cascade and contribute to coordinated regulation of blanching pathways.

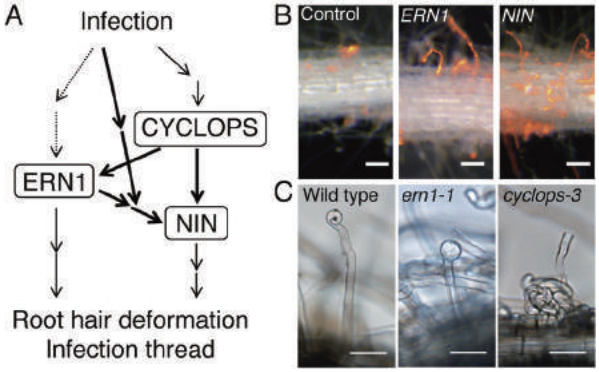

Figure 2. ERN1-mediated regulation of infection processes. (A) A model of the transcription pathway. (B) Suppression of the IT-defective *cyclops-3* phenotype by ectopic expression of *ERN1* and *NIN*. (C) Root hair deformation. Bars; 0.2 mm (B) and 50 μm (C).

II. Arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizal fungi (AMF) mainly establish symbiotic relationships with most land plants for the purpose of nutrient exchange. Many studies have revealed the regulation of processes in AMF, such as nutrient absorption from soil, metabolism and exchange with host plants, and the genes involved in that process. However, the spatial regulation of the genes within the structures comprising each developmental stage is not well understood. Accordingly, we have demonstrated the structure-specific transcriptome of the model AMF species, *Rhizophagus irregularis*. We performed an ultra-low input RNA-seq analysis, SMART-seq2, comparing five extraradical structures, germ tubes (GT), runner hyphae (RH), branched absorbing structures (BAS), immature spores (IS) and mature spores (MS) (Figure 3). Our results suggest the importance of RH in the absorption and reduction of nitrate and that of the BAS in the absorption of Pi, ammonium, and carboxylate. The enrichment of numerous GO terms involved in DNA replication and nuclear cell division in IS appears to reflect the burst of mitosis that occurs during spore maturation. *SP7*, a secreted fungal effector of *R. irregularis* was markedly expressed in MS. In addition to this, we reanalyzed the recently reported RNA-seq data comparing intraradical mycelium and arbuscule. Our analyses captured the distinct features of each structure and revealed the structure-specific expression patterns of genes related to nutrient transport and metabolism. A finding of note was that the transcriptional profiles indicated what the distinct functions performed by BAS are in nutrient absorption. These findings provide a comprehensive dataset to advance our understanding of the transcriptional dynamics of fungal nutrition in this symbiotic system.

Furthermore, we have also applied for a patent for non-symbiotic culture of AMF in cooperation with Professors Kohki Akiyama from Osaka Prefecture University, Katsuharu Saito from Shinshu University, and Tatsuya Ezawa from Hokkaido University.

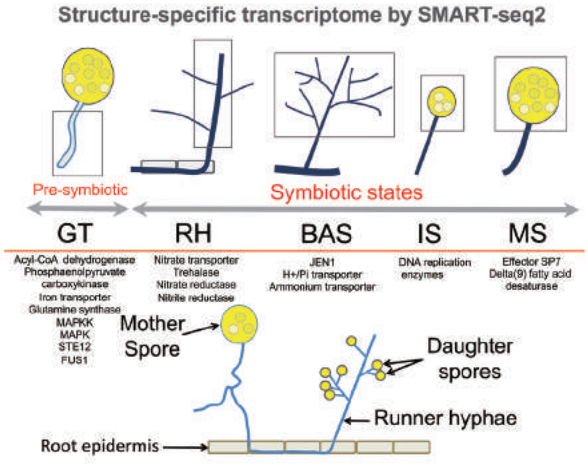

Figure 3. Five extraradical structures of R. irregularis DAOM197198; germ tubes (GT), runner hyphae (RH), branched absorbing structures (BAS), immature spores (IS) and mature spores (MS), and representative genes highly expressed in each structure detected by SMART-seq2 analysis.

III *Polygala paniculata*, a new model species for study of evolution of root nodule symbiosis

Plants exhibiting root nodule symbiosis are arranged only in only four orders of angiosperms called the monophyletic nitrogen-fixing clade. This phylogenetic relationship has raised the hypothesis which states that a predisposition facilitating acquisition of the symbiosis occurs once in a common ancestor in this clade. However, detailed evolutionary processes related to this are largely unclear. Performing comparative analyses with non-nodulating species phylogenetically related to legumes could be a better strategy for studying the evolutionary processes of nodule symbiosis.

P. paniculata is a non-nodulating plant belonging to a family that is different from legumes but is classified into the same order, Fabales (Figure 4A). It has a small body size, high fertility and a short lifecycle, whereas other families in Fabales were found to be trees (with the exception of legumes). These characteristics are advantageous when cultivating them in laboratories. Thus, we assessed whether this species was suitable as a model species for comparative studies with legumes. The estimated genome size of this species was less than that of *L. japonicus*. Therefore, the whole genome sequencing of *P. paniculata* could be determined by comparative genomics. We further developed the hairy root transformation method (Figure 4B) and optimized tissue culture conditions to regenerate individual specimens. These cultivation techniques enable gene function analyses in this species.

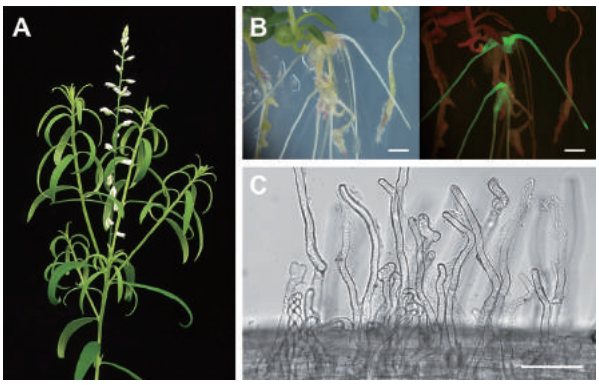

Figure 4. Characteristics of a non-leguminous plant *P. paniculata*. (A) A two months old plant. (B) Hairy roots generated from *P. paniculata* hypocotyl. (C) Deformed root hairs of *P. paniculata* inoculated with *M. loti* broad host range strain NZP2037. Bars; 2 mm (B) and 100 μm (C).

P. paniculata can interact with AM fungi, thus suggesting the presence of early signaling factors in nodule symbiosis. Deformation of root hairs was observed when they were inoculated with *Mesorhizobium loti* broad host range strain, indicating that *P. paniculata* has the potential to respond to rhizobia (Figure 4C). As such, *P. paniculata* is a good candidate as a model plant in the evolutionary study of root nodule symbiosis.

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DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY

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The Division of Evolutionary Developmental Biology was started in June 2015. We focus on the evolutionary novelties acquired by insects in order to elucidate the molecular and evolutionary mechanisms that lead to the large variety of traits that they display. Among 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) the acquisition and diversification of beetle horns.

I. Origin and diversification of insect wings

The flight organ of insects has uniquely evolved when compared to that of other various flying animals on earth,. Despite over two centuries of debate, the evolutionary origin of insect wings are still an enigma; one which we are trying to decipher it by the use of 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 research candidates in the investigation of 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. To achieve this end, 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 the functional effects of altered transcription for each of these wing genes in 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 the understanding of 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. This in turn 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 concerning the evolution of insects, such as insect wing origin. We are now conducting gene knock-out/in within various "wing" genes to identify genetic details and cell lineage analyses in *T. domestica* (Figure 1).

II. Wing color patterns and mimicry of ladybird beetles

A tremendous range of diversity in wing color patterns has evolved among insects, which in turn play various ecologically important roles such as intraspecific sexual signaling, mimesis, mimicry, and as a warning signal to 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 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.

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

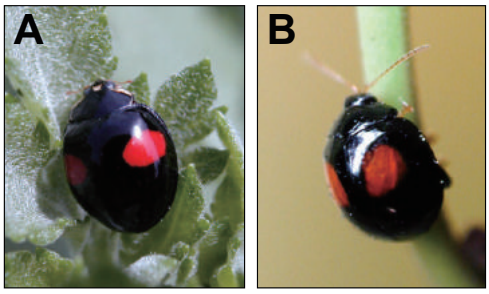

Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

Mimicry provides us with 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 synthesizes black pigment, and suppresses 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 with a focus 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 the evolution of wing color patterns in ladybird beetles. We are also establishing cryopreservation methods for germline cells in ladybird beetles to assist in 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 further facilitate this research.

In the future, we plan 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 for studying how an extreme, sex-specific morphology is formed (Figure 3, Control).

We have recently developed a larval RNAi technique in *T. dichotomus*, which allowed us to molecularly dissect the relationship between the conserved genetic pathway for sex differentiation and sexually dimorphic horn formation during post embryonic development. We systematically evaluated the function of the sex determination gene, *transformer* (*tra*) in different developmental stages, and revealed in which tissue and developmental stage the gene regulatory network for sex differentiation is activated to form sexual dimorphic horn in the head and thorax. In *T. dichotomus*, *tra* regulates sex-specific splicing of the *doublesex* pre-mRNA, and its loss of function results in sex transformation in females (Figure 3). *tra* RNAi treatments in females at early developmental stages during metamorphosis resulted in full sexual transformation, whereas no transformation is observed in the treatments at later stage. Therefore, we could estimate the onset of activation of the developmental program for the sexually dimorphic horn formation by determining the latest RNAi treatment timing when a full sexual transformation phenotype is observed. Based on this approach, we estimated that the developmental program for sexually dimorphic horn formation is activated at 29 hours after the prepupal period.

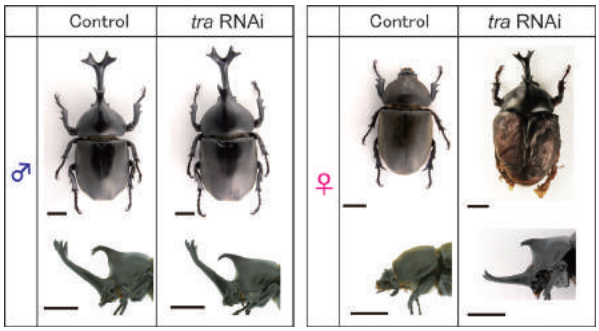

Figure 3. *tra* RNAi phenotypes. In the *tra* RNAi females, ectopic horn formation was caused by the sex transformation (masculinization). (Adapted from Morita *et al*., PLOS Genet., 15: e1008063, 2019)

We are currently focusing on this developmental stage because crucial regulatory factors for horn formation and differentiation are supposed to be activated at this stage in *T. dichotomus*. The present study provides a good starting point to unveil the gene regulatory network for sexually dimorphic horn formation and to purse the evolutionary origin of such a regulatory system.

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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 and CRISPR-Cas9 genome editing.

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 (IAGC, PLOS Biol. 2010; Shigenobu *et al*., Nature. 2000). 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) (Shigenobu & Stern, Proc. R. Soc. B 2013). 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 (Uchi *et al*., Microbes. Environ. 2019; This paper was selected as the "Most Valuable Paper of the Year 2019" in the journal, Microbes and Environments). 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.

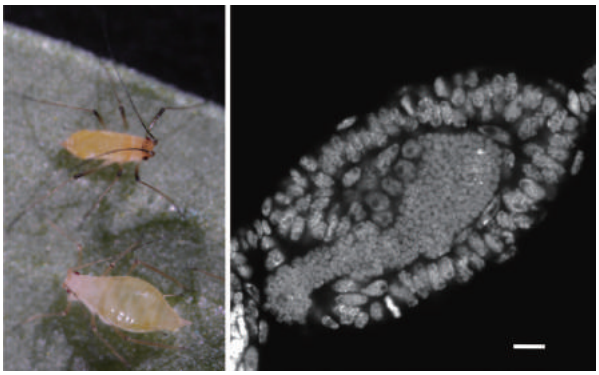

Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20 um.

Publication List

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LABORATORY OF BIORESOURCES

Specially Appointed Professor
NARUSE, Kiyoshi

Assistant Professor:	*ANSAI, Satoshi*
Research Staff:	*KANEKO, Hiroyo* *HARA, Ikuyo* *YANOGAWA, Azusa*
Visiting Scientist:	*SATO, Tadashi*
Visiting Graduate Student:	*IMAEDA YASSUMOTO, Tamiris*
Technical Assistant:	*AJIOKA, Rie* *KOIKE, Chieko* *KOIKE, Yukari* *TAKAKI, Chikako* *TESHIMA, Yuuko* *TORII, Naoko* *YAMAZAKI, Toko*
Secretary:	*SUZUKI, Tokiko*

The medaka is a small egg-laying 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 dynamics of transcriptional and chromatin accesssibility landscape during medaka development, the understanding of pigment cell differentiation using body color mutants, genome sequence of the Javanese medaka, *Oryzias javanicus* and the molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species, In addition to these activities, our laboratory was charged with the responsibility of leading the National BioResource Project Medaka (NBRP Medaka) from 2007.

I. Daynamic transcriptional and chromatin accesibikity landscape during medaka development.

A high-quality genome sequence and a variety of genetic tools are available for medaka. However, existing genome annotation is still rudimentary, as it was mainly based on computational prediction and short-read RNA-seq data. To overcome this situations, we conducted long-read RNA-seq, short-read RNA-seq, and ATAC-seq coraboration with Dr. Tu at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. This work constructed a much-improved gene model set including about 17,000 novel isoforms and identified 1600 transcription factors, 1100 long non-coding RNAs, and 150,000 potential cis-regulatory elements as well. The work provides the first comprehensive omics datasets of medaka embryogenesis. The data portal (http://tulab.genetics.ac.cn/medaka_omics) will serve as a daily reference tool for the entire medaka community.

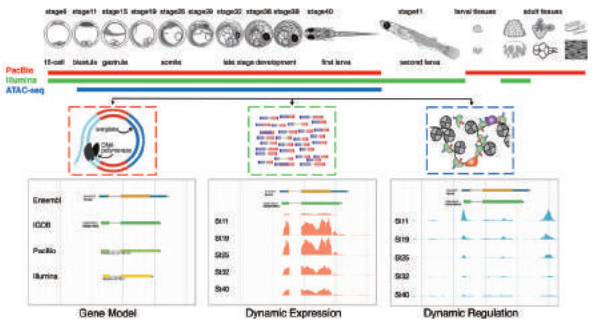

Figure 1. Overview of medaka multi-omics anslysis during development

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 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 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.

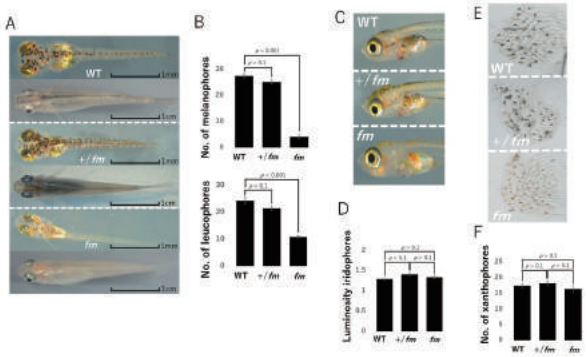

Figure 2. The *fm* mutants show a reduction in melanophore and leucophore pigmentation(A and B) and not in xanthophore and iridophore pigmentation (C, D, E and F).

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 it's species. Among these species, the Java 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, intestine and kidney, 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.

Publication List:

〔**Original papers**〕

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〔**Review articles**〕

- Murata, K., Kinoshita, M., Naruse, K., Tanaka, M., and Kamei, Y. eds. (2020). Medaka: Biology, Managements, and Experimental Protocols, Volume 2. (Wiley-Blackwell, Iowa, USA). ISBN: 978-1-119-57529-0
- Naruse, K., Takehana, Y., and Yamahira, K. (2020). Medaka and *Oryzias* species as model organisms and the current status of medaka biological resources. In Medaka: Biology, Managements, and Experimental Protocols, Volume 2, K. Murata, M. Kinoshita, K. Naruse, M. Tanaka, and Y. Kamei, eds. pp. 31-48. ISBN: 978-1-119-57529-0

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).

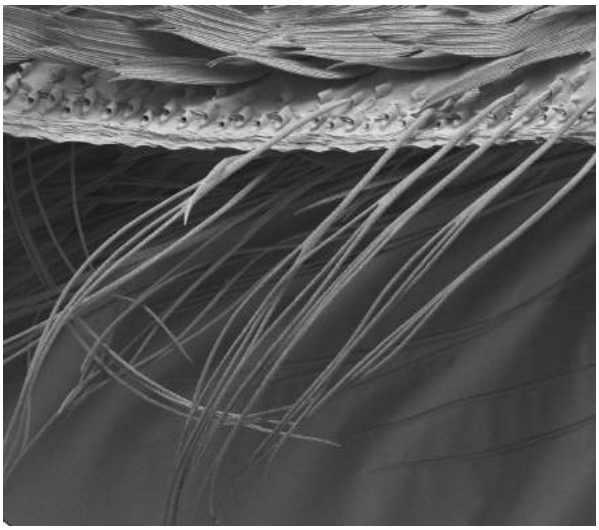

Figure 1. Scanning electron microscopy of the wing margin of *P. operculella*. Long marginal scales show branching morphology.

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).

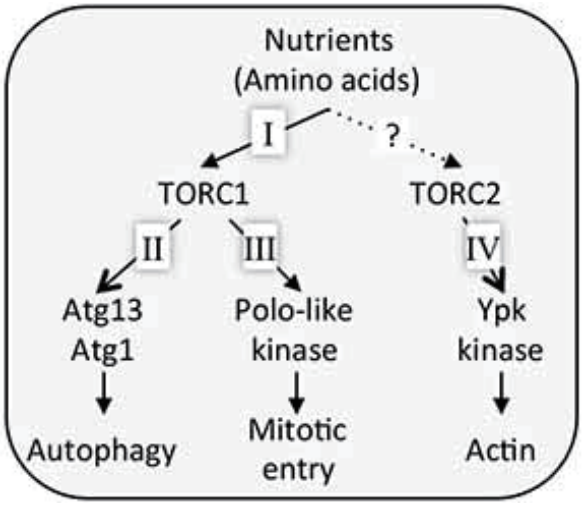

Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

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).

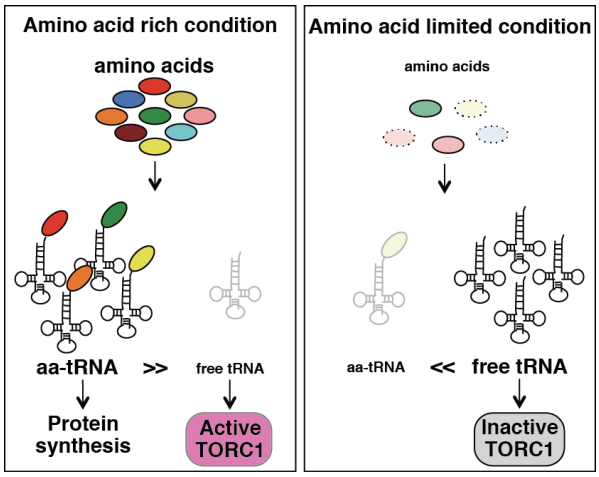

Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

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〕

● Baba, M., Tomonaga, S., Suzuki, M., Gen, M., Takeda, E., Matsuura, A., Kamada, Y., and Baba, N. (2019). 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 *15*, 423-437. doi: 10.1080/15548627.2018.1525475

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

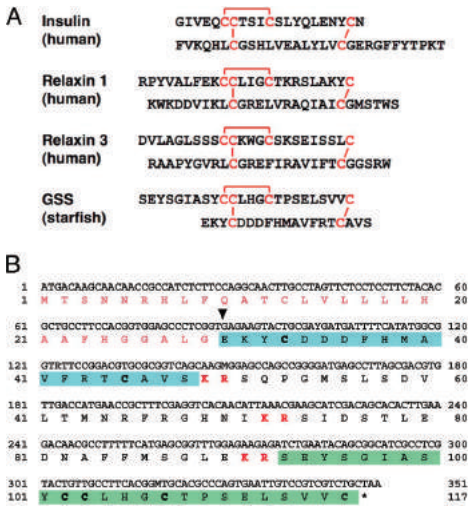

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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 this pigmentation (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. Recreating the lost morning glory

I. nil cultivars are displayed in a wide variety of flower colors: red, peach, purple, brown and white. However, just as roses do not have blue flowers, morning glories do not have yellow flowers. Yellow-flowered morning glories have been recorded in illustrations from the Edo period, but this color variety has since been lost. For this reason, it has been called the 'phantom morning glory', and many efforts have been made to reproduce it over a long period. In the yellow-flowered snapdragon, yellow pigment aurones are synthesized by the chalcone glycosyltransferase and aurone synthase genes from chalcone. Although *I. nil* produces chalcone, it lacks an ability to produce large quantities of aurones. The two snapdragon genes were introduced in the *I. nil* mutant accumulating chalcones in its cream yellow flowers. The transgenic plants expressing both genes exhibited yellow flowers; a characteristic sought for many years. The flower petals of the transgenic plants contained the snapdragon aurones and a novel acylated aurone.

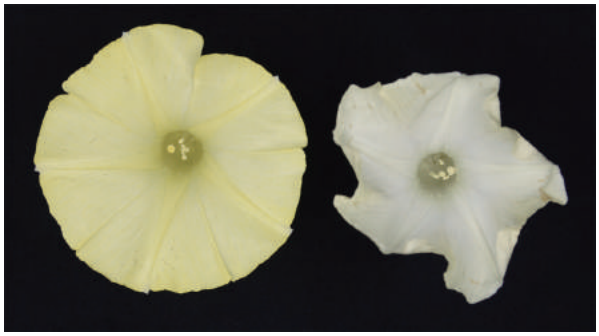

Figure 2. Flowers of the recreated 'phantom morning glory' (left), and the host plant (right). The host plant often exhibits shriveled flowers with necrotic cells, the transgenic plants produced fully opened flowers with few necrotic cells.

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, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* is one of the most popular floricultural plants in Japan, and has a 100-year history of extensive genetic studies related to it. Our collection includes 220 lines and 177,000 DNA clones. The whole genome sequence, the transcriptome sequences, as well as the end sequences of the DNA clones can be viewed via the *I. nil* genome database (http://viewer.shigen.info/asagao/index.php).

Publication List:

〔Original papers〕

- Hoshino, A., Mizuno, T., Shimizu, K., Mori, S., Fukada-Tanaka, S., Furukawa, K., Ishiguro, K., Tanaka, Y., and Iida, S. (2019). Generation of yellow flowers of the japanese morning glory by engineering its flavonoid biosynthetic pathway toward aurones. Plant Cell Physiol. *60*, 1871-1879. doi: 10.1093/pcp/pcz101
- Waki, T., Mameda, R., Nakano, T., Yamada, S., Terashita, M., Ito, K., Tenma, N., Li, Y., Fujino, N., Uno, K., Yamashita, S., Aoki, Y., Denessiouk, K., Kawai, Y., Sugawara, S., Saito, K., Yonekura-Sakakibara, K., Morita, Y., Hoshino, A., Takahashi, S., and Nakayama, T. (2020). A conserved strategy of chalcone isomerase-like protein to rectify promiscuous chalcone synthase specificity. Nat. Commun. *11*, 870. doi: 10.1038/s41467-020-14558-9

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 gain (*Lgg*). Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

The identified *LGG* gene shows similarity to RNA binding proteins. Transgenic rice lines with knock-out (KO) and over-expressing (OE) in LGG gene showed large and small seed sizes, respectively. The section of lemma KO and OE plant were observed, and their cell numbers counted (Figure2). Increased cell numbers in *Lgg* mutant have induced the *Large Grain* phenotype. RNA seq analysis revealed that expression of cell-cycle and prolification related genes was changed in transgenic plants.

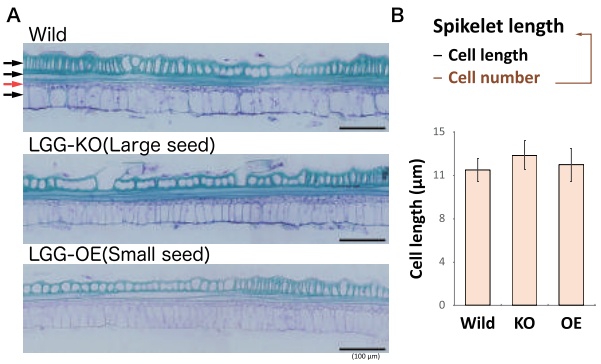

Figure 2. The section and cell number of lemma in transgenic plants. Bar = 100 μm. (A) the section of lemma in rice plants. The red arrow indicates counted cells. (B) Graph of cell number of lemma

Publication List:

〔Original papers〕

- Chiou, W.-Y., Kawamoto, T., Himi, E., Rikiishi, K., Sugimoto, M., Hayashi-Tsugane, M., Tsugane, K., and Maekawa, M. (2019). LARGE GRAIN Encodes a putative RNA-binding protein that regulates spikelet hull length in rice. Plant Cell Physiol. *60*, 503-515. doi: 10.1093/pcp/pcz014
- Nishimura, H., Himi, E., Eun, C.-H., Takahashi, H., Qian, Q., Tsugane, K., and Maekawa, M. (2019). Transgenerational activation of an autonomous DNA transposon, Dart1-24, by 5-azaC treatment in rice. Theor. Appl. Genet. *132*, 3347-3355. doi: 10.1007/s00122-019-03429-7
- Nishimura, H., Himi, E., Rikiishi, K., Tsugane, K., and Maekawa, M. (2019). Establishment of nDart1-tagged lines of Koshihikari, an elite variety of rice in Japan. Breed. Sci. *69*, 696-701. doi: 10.1270/jsbbs.19049

LABORATORY OF BIOLOGICAL DIVERSITY

SAKUTA Group

Assistant Professor: *SAKUTA, Hiraki*
Technical Assistant: *ISOSHIMA, Yoshiko*
KODAMA, Akiko

The homeostatic osmoregulation of body fluids (such as plasma and cerebrospinal fluid (CSF)) is vital to life. This is because substantial changes in cell volumes due to hypertonicity or hypotonicity cause irreversible damage to organs and lead to lethal neurological trauma. Water deprivation (loss of water from the body) elevates the concentration of Na^+ ($[Na^+]$) and osmolality in body fluids. Animals exhibit prominent and effective responses to water deprivation, including behavioral responses, such as inducing water intake and avoiding sodium (Na), along with vasopressin-induced reductions in urine volumes. The aim of our research group is to reveal the brain systems for body-fluid homeostasis.

I. Thirst control by Na_x and TRPV4

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

We recently demonstrated that Na_x signals are also involved in the control of water intake behavior. Our pharmacological experiments suggested that Na_x signals led to the activation of neurons bearing TRPV4 by using epoxyeicosatrienoic acids (EETs) as gliotransmitters to stimulate water intake. This year, we performed selective lesions of individual sCVOs in wild-type (WT) mice and the site-directed rescue of *Na$_x$* expression in *Na$_x$* knockout (*Na$_x$*-KO) mice. These experiments revealed that the Na_x channel in the OVLT functions as a $[Na^+]$ sensor for the control of water intake behavior. Direct measurements of 5,6-EET and 8,9-EET in the OVLT revealed that EET levels were indeed increased two-fold by water deprivation for two days in WT, but not *Na$_x$*-KO mice. This indicates that EETs were Na_x-dependently produced in the OVLT in response to increases in $[Na^+]$ in body fluids. More importantly, the ICV injection of 5,6-EET at the same level was effective in inducing water intake.

The signaling mechanisms in the OVLT for water-intake induction by increases in $[Na^+]$ in body fluids are presented in Figure 1. When $[Na^+]$ in plasma and CSF increases, Na_x channels in glial cells in the OVLT are activated, leading to the synthesis of EETs in Na_x-positive glial cells. EETs released from Na_x-positive glial cells function as gliotransmitters to activate neurons bearing TRPV4 channels in the OVLT, which are involved in the stimulation of water-intake behavior.

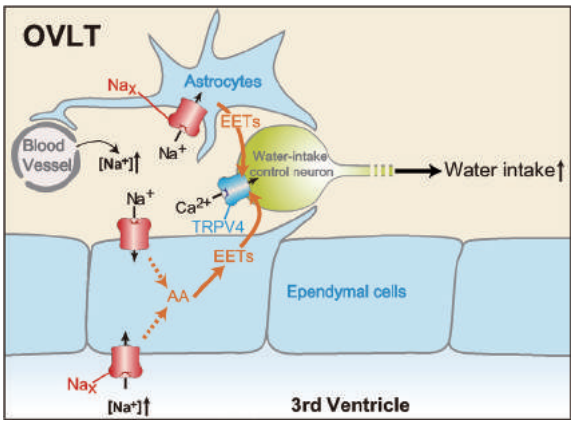

Figure 1. Proposed mechanisms for water intake induction by increases in $[Na^+]$ in body fluids via Na_x activation in the OVLT. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

Water intake by *Na$_x$*-KO mice after the ICV injection of hypertonic NaCl solution was small, but still approximately half that by WT mice and, noteworthily, significantly higher than that by *Na$_x$*-KO and WT mice after the ICV injection of an equimolar hypertonic sorbitol solution. These findings suggest the existence of another unknown $[Na^+]$ sensor and osmosensor. In order to identify the novel sensors involved in water intake control, we performed RNA-seq analysis of OVLT and identified several candidates. We are now examining the functional roles of these candidates in water intake.

Publication List:

〔Original papers〕

- 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. (2019). $[Na^+]$ increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASIC1a. Neuron *101*, 60-75. doi: 10.1016/j.neuron.2018.11.017
- Winkelman, B.H.J., Howlett, M.H.C., Hölzel, M.-B., Joling, C., Fransen, K.H., Pangeni, G., Kamermans, S., Sakuta, H., Noda, M., Simonsz, H.J., McCall, M.A., De Zeeuw, C.I., and Kamermans, M. (2019). Nystagmus in patients with congenital stationary night blindness (CSNB) originates from synchronously firing retinal ganglion cells. PLoS Biol. *17*, e3000174. doi: 10.1371/journal.pbio.3000174

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

- Sakuta, H., Lin, C.-H., Yamada, M., Kita, Y., Tokuoka, S.M., Shimizu, T., and Noda, M. Na_x-positive glial cells in the organum vasculosum laminae terminalis produce epoxyeicosatrienoic acids to induce water intake in response to increases in $[Na^+]$ in body fluids. Neurosci. Res. 2019 May 28. doi: 10.1016/j.neures.2019.05.006

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*.).

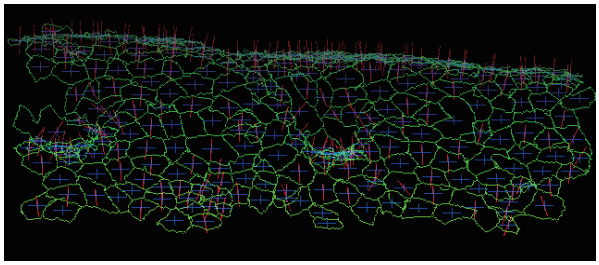

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

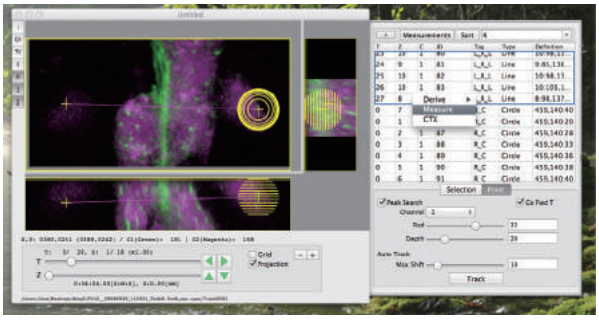

Figure 2. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

Publication List:

〔Original papers〕

- Fujita, I., Shitamukai, A., Kusumoto, F., Mase, S., Suetsugu, T., Omori, A., Kato, K., Abe, T., Shioi, G., Konno, D., and Matsuzaki, F. (2019). Endfoot regeneration restricts radial glial state and prevents translocation into the outer subventricular zone in early mammalian brain development. Nat. Cell Biol. *22*, 26-37. doi: 10.1038/s41556-019-0436-9
- Furutani, M., Hirano, Y., Nishimura, T., Nakamura, M., Taniguchi, M., Suzuki, K., Oshida, R., Kondo, C., Sun, S., Kato, K., Fukao, Y., Hakoshima, T., and Morita, M.T. (2020). Polar recruitment of RLD by LAZY1-like protein during gravity signaling in root branch angle control. Nat. Commun. *11*, 76. doi: 10.1038/s41467-019-13729-7

LABORATORY OF BIOLOGICAL DIVERSITY†

OHTA Group

Specially Appointed Assistant Professor:
OHTA, Yusaku

Image analysis is an important element in understanding life science. It makes it possible to quantify phenomena by extracting meaningful information from a large amount of images and then appropriately expressing said information. In recent years, machine learning, including deep learning, has changed image analysis in the field of biology. In keeping with this, I am currently using image analysis technology to elucidate the principles of embryo development and to provide comprehensive imaging support in life science.

I. Elucidation of the principle of collective migration of cells that maintain the order of embryonic development.

During organism morphogenesis, three-dimensional remodeling of tissues by cell migration is essential. While individual cell motility depends on extracellular signals, cell-cell adhesion is maintained, thereby controlling highly coordinated cell motility. To elucidate the principle of such complicated embryogenesis, it is necessary to understand the cell dynamics of the whole embryo with single cell resolution. To this end, I am conducting research using the following three technologies.

The first is a four-dimensional cell tracking analysis that automatically tracks all cells in the early embryo. This makes it possible to treat individual cell dynamics of the whole embryo as digital information. The second is the automation of visualization of cell dynamics information from large-scale image data. Image data displaying an entire embryo can exceed terabytes, which in turn exceeds much analysis can be conducted by the visual and manual labor of researchers. This automation makes it easier to understand the behavior of tens of thousands of cells. The third technology is the identification of key cells that control morphogenesis by machine learning. This enables objective and quantitative analysis instead of subjective analysis that relies on the researchers' preconceptions .

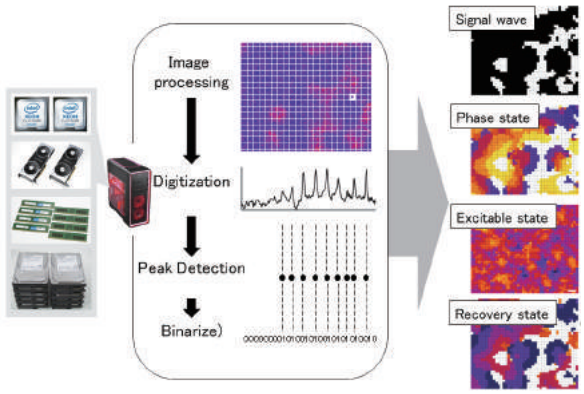

Figure 1. Example of automated image analysis

II. Research support by image analysis

The development of imaging technology has been remarkable within life science research, and many researchers are now able to easily acquire large and complex sets of image data. However, image analysis is still a hurdle for researchers, and it often creates bottlenecks in research. In order to solve this problem, I provide research support based on the following three themes.

The first theme is quantitative image analysis based on a wealth of knowledge in imaging and statistics. For many researchers, the method of evaluating information contained in images is limited to qualitative and subjective types. Correct analysis based on knowledge of imaging and statistics supports quantitative and objective analysis. The second theme is the active utilization of image analysis technology via the application of machine learning, including deep learning. In recent years, the development of machine learning has been remarkable, and with a little training, it is possible to simplify analysis that is difficult to achieve with conventional image analysis technology. The third theme is relates to the publication of explanations of image analysis to researchers in an easy-to-understand manner on the web. The contents of which range from the principles of image analysis methods to the use of image analysis software and plug-ins.

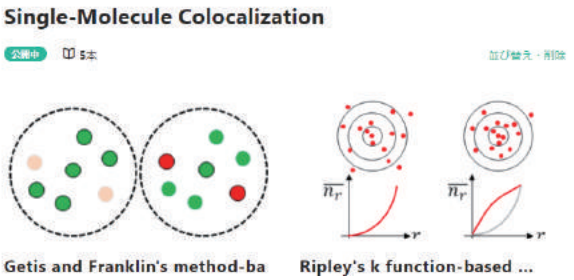

Figure 2. A website with topics related to image analysis

Publication List:

〔Original paper〕

● Yagi, H., Yagi-Utsumi, M., Honda, R., Ohta, Y., Saito, T., Nishio, M., Ninagawa, S., Suzuki, K., Anzai, T., Kamiya, Y., Aoki, K., Nakanishi, M., Satoh, T., and Kato, K. (2020). Improved secretion of glycoproteins using an N-glycan-restricted passport sequence tag recognized by cargo receptor. Nat. Commun. *11*, 1368. doi: 10.1038/s41467-020-15192-1

LABORATORY OF BIOLOGICAL DIVERSITY†

SHINOMIYA Group

Specially Appointed Assistant Professor: SHINOMIYA, Ai
Technical Assistant: TSURUTA, Emiko

The natural environment, which incorporates phenomena such as day length, solar radiation, temperature, and precipitation, generates seasonal changes that affect organisms. Although animals alter their physiology and behavior in response to seasonal changes in their environment, the mechanism of seasonal adaptation remains largely unknown.

Medaka (*Oryzias latipes*) provide an excellent model to study these mechanisms because of their rapid and obvious seasonal responses. In addition, it is also possible to apply transgenic and genome-editing approaches when researching them, as well as reference genome sequences. In keeping with this, we are currently using Medaka to study the molecular mechanisms of seasonal adaptation, as well as the relationship between seasonal information from the environment and the organism's response.

I. Underlying mechanisms that define critical day length and temperature

Most animals living outside the tropics reproduce only during a particular season of the year. These are called seasonal breeders, and it is well established that day length is a crucial cue for reproduction in many of them. In addition, it has been demonstrated that temperature changes are also important for them in the how they detect seasons. However, it remains unknown how animals measure seasonal changes in relation to these environmental factors.

Applying forward genetic approaches, we examined critical day length in reproduction (*i. e.*, the duration of lighting time required to cause gonadal development) using various Medaka populations, that originated in various latitudes throughout Japan to elucidate these mechanisms (Figure 1).

Geographical variation critical day length was detected, and populations from lower latitudes indicated a shorter critical day length (Figure 1). To identify the genes governing critical day length, quantitative trait loci (QTL) analysis was conducted using F_2 offspring derived from crosses between

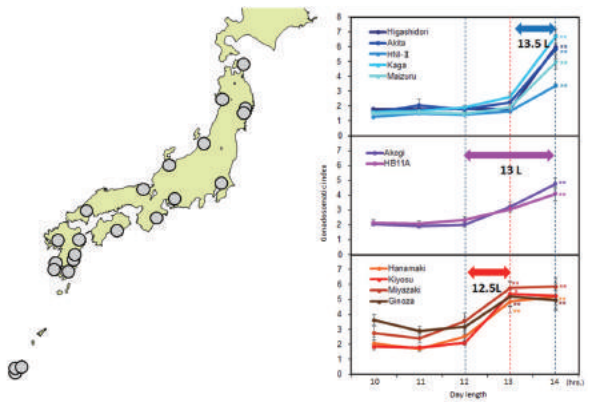

Figure 1. Medaka populations used in our study (left). Differences of critical day length among medaka populations (right).

populations experiencing different critical day length. We thus identified significant QTLs using Restriction-site Associated DNA (RAD) markers (Figure 2).

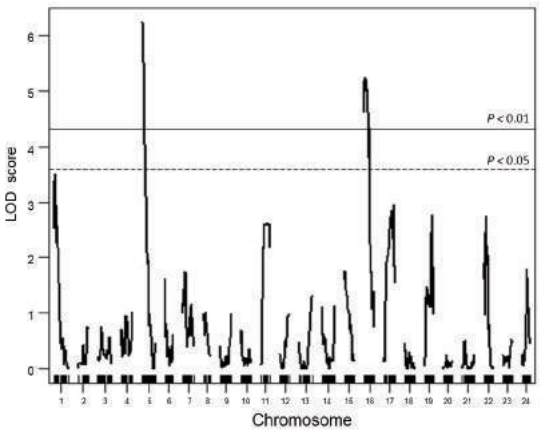

Figure 2. Result of QTL analysis for critical day length.

Whole-genome re-sequencing in various medaka populations experiencing different critical day lengths were conducted. We have identified potential candidate genes that define the critical day length by analyzing this genome sequencing data.

We also performed experiments to identify critical temperature, and subsequently detected geographical variation among Medaka populations. Significant QTLs for critical temperature have been detected from the genetic analysis that was performed.

II. Analysis of the relationship between breeding rhythms and environmental information

Organisms exhibit various scales of rhythm, ranging in seconds to years. On the other hand, the natural environment provides the rhythmic changes concerning organisms. However, the quantitative relationship between the information on environmental factors and biological rhythms is poorly understood.

We conducted a linear regression analysis regarding annual rhythms in gonadal development using data pertaining to the annual changes in the gonadal size of the medaka and environmental information in the experimental field. The regression model explained which environmental factors contributed to the seasonal change in medaka gonads and to what extent they contributed to this change.

Publication List:

〔Original papers〕

● Nakane, Y., Shinomiya, A., Ota, W., Ikegami, K., Shimmura, T., Higashi, S., Kamei, Y., and Yoshimura, T. (2019). Action spectrum for photoperiodic control of thyroid-stimulating hormone in Japanese quail (*Coturnix japonica*). PLoS One *14*, e022106. doi: 10.1371/journal.pone.0222106

● Nakayama, T., Shimmura, T., Shinomiya, A., Okimura, K., Takehana, Y., Furukawa, Y., Shimo, T., Senga, T., Nakatsukasa, M., Nishimura, T., Tanaka, M., Okubo, K., Kamei, Y., Naruse, K., and Yoshimura, T. (2019). Seasonal regulation of the lncRNA LDAIR modulates self-protective behaviours during the breeding season. Nat. Ecol. Evol. *3*, 845-852. doi: 10.1038/s41559-019-0866-6

LABORATORY OF BIOLOGICAL DIVERSITY

YAMASHITA AND OTSUBO Group

Specially Appointed Associate Professor
YAMASHITA, Akira

Specially Appointed Assistant Professor: *OTSUBO, Yoko*
Technical Assistant: *NAKADE, Atsuko*
Secretary (Senior Specialist): *SAKAGAMI, Mari*

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 based on this information. In response to ambient conditions, germ cells producing sperm and eggs begin halving their number of chromosomes during a special kind of cell division called meiosis; a process 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. In addition, we have started to study cellular responses to a novel stress: atmospheric pressure plasma.

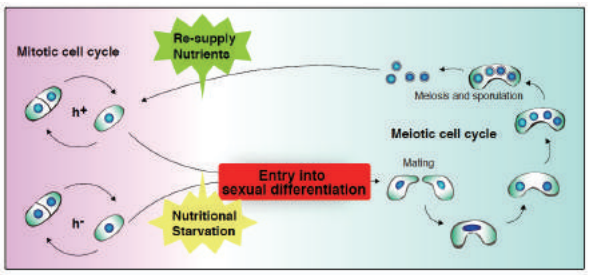

Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting 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. To achieve this, 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 starved conditions.

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich mediums in 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*, which stands for hypermating and temperature-sensitive growth. We cloned the responsible genes and found that five of the eight responsible genes 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 the expression of tRNA precursors decreases upon nitrogen starvation occurring. Furthermore, overexpression of tRNA precursors prevents TORC1 downregulation in response to 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).

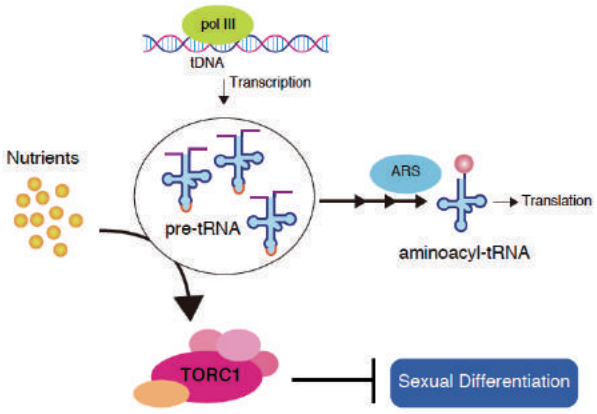

Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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 by repeated hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machin-

ery. Mmi1 also induces the formation of facultative heterochromatin 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. As such, multi-layered suppression of meiotic genes by Mmi1 is vital for mitotic growth (Figure 3).

III. Cellular responses to atmospheric pressure plasma

Plasma, which is the fourth state of matter after solid, liquid and gas states, is ionized or electrically charged gas. It has been known that plasma irradiation induces a wide variety of effects on living organisms. Recently, atmospheric pressure plasma has been used in various fields including medicine and agriculture. However, detailed mechanisms underlying responses to plasma irradiation remains unknown. Accordingly, we are addressing molecular mechanisms of cellular responses to plasma by using the fission yeast *S. pombe*. To this end, we have developed plasma irradiation devices that enable control of gas temperature in collaboration with National Institute for Fusion Science.

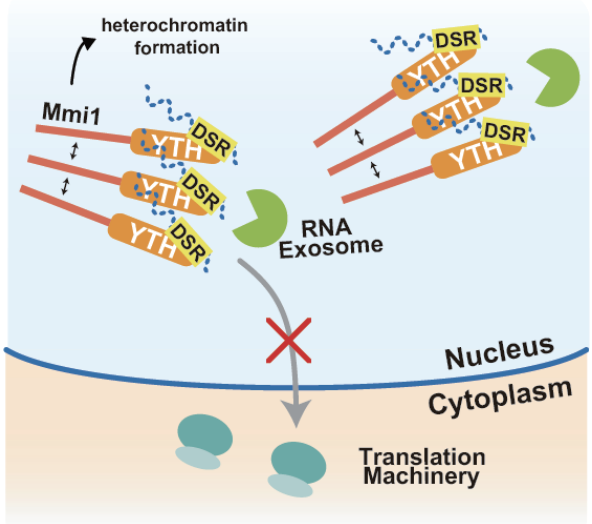

Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

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NINS Astrobiology Center

The Astrobiology Center (ABC) was established in 2015 to promote interdisciplinary studies including astronomy, earth science, and biology. Our ultimate goal is to find a so called 'Second Earth' and other extraterrestrial life on this planet. Astronomers and earth scientists in the Exo-Planet Search Project and Astrobiology Instrument Project at the main office in Tokyo are now discovering habitable planets around the nearest stars using the latest observation technologies. To support these observation projects, biologists from NIBB participating in the Exo-Life Search Project are investigating life on Earth to predict the biosignature of hypothetical life on the aforementioned 'Second Earth'.

This year, ABC (jointly with NAOJ) hosted the 4th 'In the Spirit of Lyot' international symposium in Tokyo, where the international research community found new ways to work together toward direct imaging of the 'Second Earth'. The solar coronagraph, a telescopic attachment for blocking bright light from the Sun, was invented by the namesake of this symposium, Bernard Lyot, in 1931. Stellar coronagraphs enable high contrast imaging of Earth-like planets around Sun-like stars in combination with adaptive optics and other advanced observation technologies.

Figure 1. Lyot 2019 Poster

Laboratory of Biological Diversity

TAKIZAWA Group

Specially Appointed Associate Professor
TAKIZAWA, Kenji

The Takizawa group operating out of ABC is currently studying environmental responses of photosynthesis in order to predict photosynthetic apparatus of 'Alien' plants under extreme conditions on the 'Second Earth'.

Remote sensing of vegetation red-edge

One of the most plausible biosignatures on habitable exoplanets is a specific reflection pattern on the land surface named 'red-edge' that is caused by land vegetation. Red-edge appears on Earth between red light which is absorbed by photosynthetic pigments, and near infrared radiation (NIR), which is reflected via leaf tissue structure. Vegetation indexes calculated from reflectance in red and NIR are roughly related to photosynthetic capacity. Remote sensing by drone-based, multiband sensors revealed light reflection properties of plants growing in various habitats.

Floating plants in water world

The light reflection properties peculiar to land vegetation cannot be formed by algae and plants under water. Even though aquaplanet is rare within the universe, most of the water-containing planets are considered 'ocean planets', which are filled with a substantial amount of water. If there are no continents, detection of vegetation red-edge is unlikely. We proposed there was a possibility remaining that a strong biosignature could be detected on 'water planets' due to extensive flourishing of drifting algae and floating plants. Some species of aquatic plants have sponge-like floating leaves and thus reflect NIR in a manner comparable to land vegetation.

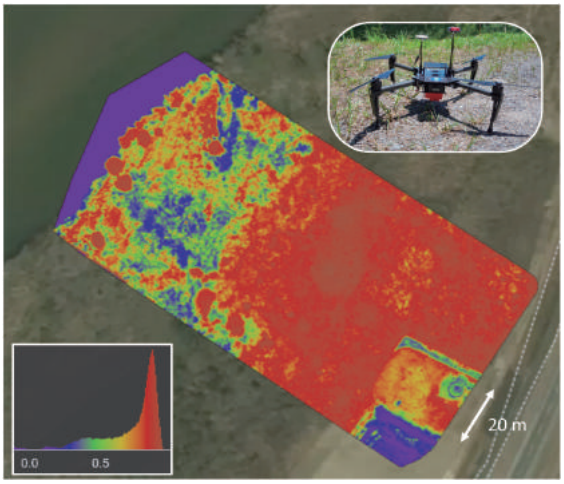

Figure 2. Normalized difference vegetation index (NDVI) survey for grassland on a riverbank. NDVI is a graphical indicator for green vegetation calculated from light reflectance in Red and NIR as (NIR-Red)/(NIR+Red). Grass covered area showed high NDVI (red color) in contrast with low NDVI (blue color) for water (upper left) and soil (lower right) area. Multiband images were obtained by drone-based sensors (pictured at upper right).

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.

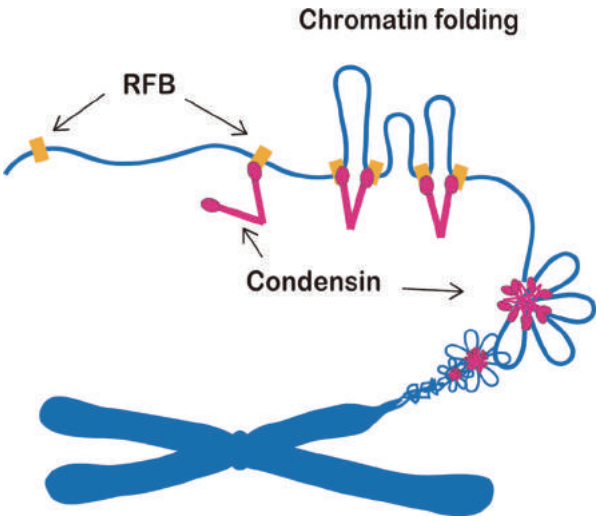

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

FUJITA Group

Assistant Professor: *FUJITA, Hironori*
Technical Assistant: *NIWA, Yoshimi*

In nature, a variety of patterns, such as the galaxy and the snowflake, are found on a wide range of spatiotemporal scales, and they are generated in a self-organizing manner. Particularly in living organisms, such self-organization of spatiotemporal patterns is both remarkable and essential. Therefore, we aim to elucidate the mechanism of generation and control of self-organized patterns in living systems with a particular focus on plants using both mathematical and computational approaches.

I. Spatial regulation of resource allocation in response to nutritional availability

It is critical for living organisms to appropriately allocate resources among its organs, or within a specific organ, because available resources are generally limited. For example, in response to the nutritional environments of their soil, plants regulate resource allocation in their roots in order to plastically change their root system architecture (RSA), so they can efficiently absorb nutrients (Figure 1A). However, it is still not understood why and how RSA is adaptively controlled. Therefore, we modeled and investigated the spatial regulation of resource allocation by focusing on RSA in response to nutrient availability, and provided analytical solutions to the optimal strategy in the case of simple fitness functions (Fujita *et al*., J. Theor. Biol. 2020). First, we showed that our model could explain the experimental evidence indicating that root growth is maximized at the optimal nutrient concentration under homogeneous conditions. Next, we extended our model to incorporate the spatial heterogeneity of nutrient availability. This extended model revealed that growth suppression by systemic control is required for adaptation to high nutrient conditions, whereas growth promotion by local control is sufficient for adaptation to low-nutrient environments (Figure 1B). This evidence indicates that systemic control can be evolved in the presence of excessive amounts of nutrition, consistent with the 'N-supply' systemic signal that is observed experimentally. Furthermore, our model can also explain various experimental results using nitrogen nutrition, and provides a theoretical basis for understanding the spatial regulation of adaptive resource allocation in response to nutritional environment.

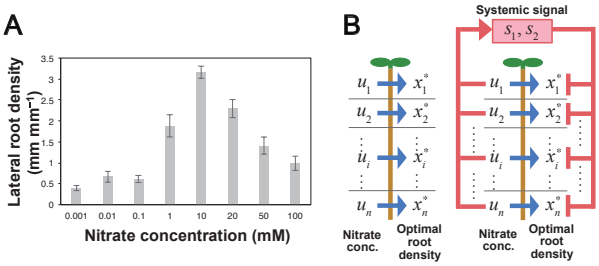

Figure 1. (A) Root growth in the homogeneous availability of nitrogen nutrition. (B) Schematic representation of the spatial regulation of the optimal resource allocation in response to nutrient availability. (left) In the low nutrient availability, the optimal root density is promoted by nutrient through local control (blue arrows). (right) By contrast, in addition to local control, is suppressed through systemic control (denoted in red) under high nutrient availability.

II. Phyllotaxis pattern formation

Phyllotaxis, the beautiful geometry of plant-leaf arrangement around the stem, has long attracted attention from researchers of biological-pattern formation. Many mathematical models, as typified by those of Douady and Couder (alternate-specific form, DC1; more generalized form, DC2), have been proposed for phyllotactic patterning, mostly based on the notion that a repulsive interaction between leaf primordia spatially regulates primordium initiation. In the framework of DC models, which assume that each primordium emits a constant power that inhibits new primordium formation, and that this inhibitory effect decreases with distance, the major (but not all) types of phyllotaxis can be manifested as a stable pattern. Orixate phyllotaxis, which has a tetrastichous alternate pattern with a four-cycle sequence of the divergence angle, is an interesting example of an unaddressed phyllotaxis type. We examined DC models regarding the ability to produce orixate phyllotaxis and found that model expansion through the introduction of primordial age-dependent changes of the inhibitory power is essential for the establishment of orixate phyllotaxis (Yonekura *et al*., PLoS Comput. Biol. 2019). The simulation results obtained using the expanded version of DC2 (EDC2) fitted well the natural distribution of phyllotactic patterns. Our findings imply that changing the inhibitory power is generally an important component of the phyllotactic patterning mechanism.

Publication List:

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- Fujita, H., Hayashi-Tsugane, M., and Kawaguchi, M. (2020). Spatial regulation of resource allocation in response to nutritional availability. J. Theor. Biol. *486*, 110078. doi: 10.1016/j.jtbi.2019.110078
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Plants and algae have a great capacity to acclimate themselves to changing environments. We are interested in these acclimation processes, and how they efficiently yet safely harness sunlight for photosynthesis under fluctuating light conditions in particular. 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 this model green alga to various phytoplankton, including a symbiotic dinoflagellate known to associate with coral and sea anemones, Symbiodiniaceae, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.

I. Amphipol-associated purification method for the highly active and stable photosystem II

Photosystem II (PSII) splits water and drives electron transfer to plastoquinone via photochemical reactions using light energy. It is surrounded by light-harvesting complex II (LHCII) to form the PSII-LHCII supercomplex. However, a complete characterization of its structure and function is hampered due to instability of the complex in the presence of detergent. To overcome this problem, we developed a new procedure for purifying the PSII-LHCII supercomplexes of *Chlamydomonas reinhardtii* by employing amphipol A8-35 (Watanabe, A., *et al*. FEBS Lett. *593*: 1072-1079). The obtained supercomplexes showed little LHCII dissociation even 4 days after purification. Oxygen-evolving activity was retained within amphipol when the extrinsic polypeptides were kept associated by betaine. Electron microscopy revealed that this method also improved structural uniformity and that the major organization was $C_2S_2M_2L_2$.

II. Structure of light harvesting for photosystem II in green algae

In *C. reinhardtii*, LHCII molecules associate with PSII to form various supercomplexes, including the $C_2S_2M_2L_2$ type; the largest PSII–LHCII supercomplex in algae and plants that is presently known. We reported high-resolution cryo-electron microscopy (cryo-EM) maps and structural models of the $C_2S_2M_2L_2$ and C_2S_2 supercomplexes from *C. reinhardtii* (Figure 1) (Sheng, X., Watanabe, A., *et al*. Nature Plants, 5: 1320-1330). The C_2S_2 supercomplex contained an LhcbM1–LhcbM2/7–LhcbM3 heterotrimer in the strongly associated LHCII, and the LhcbM1 subunit assembled with CP43 through two interfacial galactolipid molecules. The loosely and moderately associated LHCII trimers interacted closely with the minor antenna complex CP29 to form an intricate subcomplex bound to CP47 in the $C_2S_2M_2L_2$ supercomplex. A notable direct pathway was established for energy transfer from the loosely associated LHCII to the PSII reaction centre, as well as several indirect routes. Structure-based computational analysis concerning the excitation energy transfer within the two supercomplexes provided detailed mechanistic insights into the light-harvesting process in green algae.

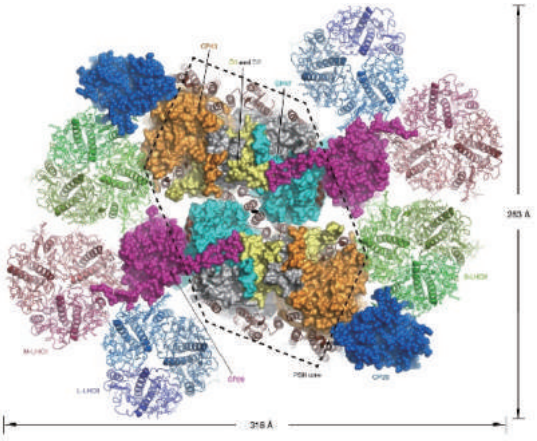

Figure 1. Top view of the $C_2S_2M_2L_2$ supercomplex from the stromal side. The central elliptical symbol indicates the C_2 axis running perpendicular to the membrane plane. Sheng, Watanabe *et al*. (2019) Nature Plants, *5*: 1320-1330.

III. Regulation of light-inducible photoprotection mechanism, qE, in green algae

Light is essential for photosynthesis, but the amounts of light that exceed an organism's assimilation capacity can result in oxidative stress and even cell death. Plants and microalgae have developed a photoprotective response mechanism, qE, that dissipates excess light energy as thermal energy. In the green alga *C. reinhardtii*, qE is regulated by light-inducible photoprotective proteins. However, the pathway from light perception to qE until now has remained unknown. We discovered that the transcription factors CONSTANS and Nuclear transcription Factor Ys (NF-Ys) form a complex that governs light-dependent photoprotective responses in *C. reinhardtii* (Tokutsu, R., *et al*. Nature Commun., *10*: 4099). The qE responses did not occur in CONSTANS or NF-Y mutants. The signal from light perception to the CONSTANS/NF-Ys complex was directly inhibited by the SPA1/COP1-dependent E3 ubiquitin ligase. This negative regulation mediated by the E3 ubiquitin ligase and the CONSTANS/NF-Ys complex was common to photoprotective response in algal photosynthesis and flowering in plants.

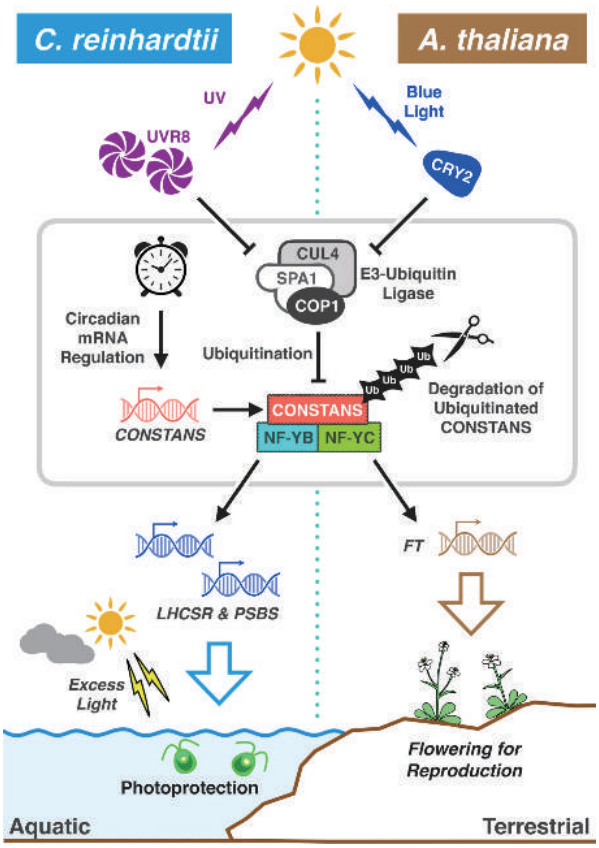

Figure 2. Hypothetical signal transduction pathways and physiological functions of CONSTANS/NF-YB/NF-YC-dependent control of the target genes in *C. reinhardtii* and *A. thaliana*. Tokutsu *et al*. (2019) Nature Commun., *10*: 4099.

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

Reef-building corals form an obligate symbiotic relationship with dinoflagellates from the family Symbiodiniaceae. Most coral species recruit algal symbionts from the environment. However, it has remained unknown how they encounter each other. We focused on green fluorescence protein (GFP)-associated fluorescence, which is commonly seen in coral (Figure 3), and examined whether it attracts motile algae. We first examined their phototaxis behavior and found that symbiotic algae show positive and negative phototaxis mostly toward strong blue and weak green light, respectively. Attraction shown by algae towards green fluorescence was observed by using both a live coral fragment and an artificial green-fluorescence dye, but only under blue light conditions *i.e*. the wavelength that induces green florescence. We also showed that traps painted with a green fluorescent dye attracted symbiotic algae 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. USA *116*, 2118-2123).

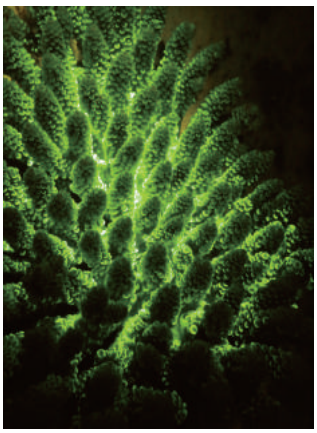

Figure 3. Emission of green fluorescence from corals under the exposure to blue light.

Publication List:

〔Original Papers〕

- Aihara, Y., Fujimura-Kamada, K., Yamasaki, T., and Minagawa, J. (2019). Algal photoprotection is regulated by the E3 ligase CUL4-DDB1^{DET1}. Nat. Plants *5*, 34-40. doi: 10.1038/s41477-018-0332-5
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〔Original paper (E-publication ahead of print)〕

- Negi, S., Perrine, Z., Friedland, N., Kumar, A., Tokutsu, R., Minagawa, J., Berg, H., Barry, A., Govindjee, and Sayre, R. Light-regulation of light harvesting antenna size substantially enhances photosynthetic efficiency and biomass yield in green algae. Plant J. 2020 Mar 17. doi: 10.1111/tpj.14751

DIVISION OF PLANT ENVIRONMENTAL RESPONSES

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Plant organs have the ability to sense various vectorial stimuli such as light, humidity and gravity as well as reorient their growth direction so as to be in a suitable position to survive and acclimate to their environment. These types of responses are referred to as tropisms. Gravitropism is a major determinant in the directing of plant organ growth angles. In gravity sensing cells (statocytes), plastids accumulating starch at a high-density relocate toward the direction of gravity. Amyloplast relocation serves as a physical signal trigger for biochemical signal transduction, which in turn leads to the regulation of polar auxin transport necessary for change in the direction that a given plant is growing. The above points raise the following important questions: 1) How is amyloplast relocation converted into a biochemical signal? 2) How do signals affect directional plant growth?

In keeping with this, we aim to understand the detailed molecular mechanism of gravity signaling by applying genetic and molecular biological approaches that use the model plant *Arabidopsis thaliana*.

I. Molecular mechanisms associated with gravity signaling

1-1 LZYs and their interactors RLDs are involved in gravitropism

*LAZY1 f*amily genes are involved in gravitropism in many plant species. We previously found that *LAZY1-LIKE (LZY) 1*, *LZY2*, and *LZY3,* are required for gravity signal transduction in statocytes following amyloplast relocation, which leads to the determination of the growth angle of roots and shoots in Arabidopsis. LZY2 and LZY3 are major contributors to root gravitropism (Figure 1A). *LAZY1* family genes encode plant specific proteins with no domain in which the function is inferable. To elucidate the molecular function of these LZY proteins, we performed yeast two-hybrid screens and immunoprecipitation coupled with mass spectrometry to identify their interactors. We found that LZY2 and LZY3 interact with RCC1-like domain (RLD) proteins. There are eight *RLD* family genes in *Arabidopsis* genome. *RLD* family genes are conserved among land plants and share a similar domain combination containing a pleckstrin homology (PH) domain, regulator of chromosome condensation 1 (RCC1)-like motif repeats, a Fab1/YGL023/Vps27/EEA1 (FYVE) domain, and a Brevis radix (BRX) domain. *Promoter:GUS* analysis indicates that four *RLD* genes are expressed in the root caps and vascular tissues of primary roots and lateral roots (Figure 1B). While *lzy* multiple mutants exhibit the disturbed gravity setpoint angle both in primary roots and shoots, *rld1 rld4* double mutants display a similar root phenotype to that found in *lzy* multiple mutants (Figure 1A, C). *rld1 rld4* double mutants fail to establish the asymmetric auxin distribution in roots as observed in *lzy* multiple mutants. These findings suggest that both *RLD*s and *LZY*s play an important role in gravity signaling.

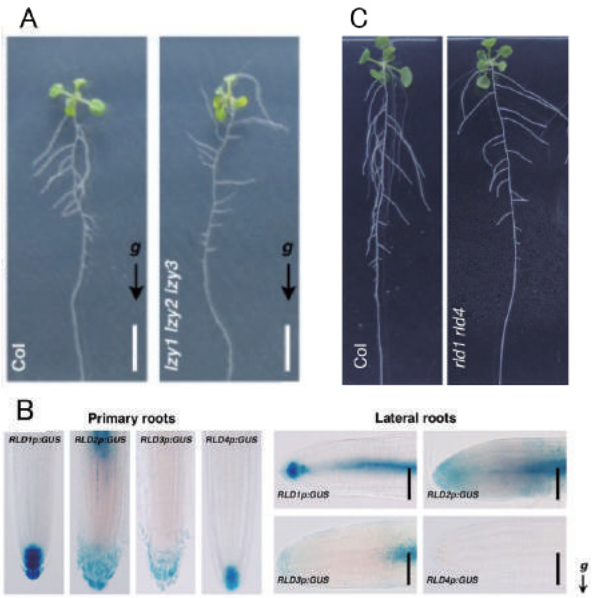

Figure 1. Role of *RLDs* and *LZYs* in gravitropic setpoint angle. **A and C**. Growth angle of lateral roots in *lzy1 lzy2 lzy3* and *rld1 rld4* mutants. Arrows indicate the direction of gravity. **B**. Histochemical assay for *RLD* gene promoter activities. GUS staining of the primary and lateral roots in *RLD1p, RLD2p, RLD3p or RLD4p:GUS* lines are shown.

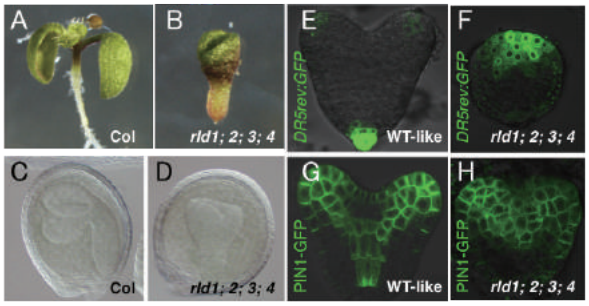

Figure 2. Loss-of-function of *RLD* genes disrupt the patterns of PIN1-GFP expression and auxin distribution. **A and B**. Seven-day-old seedlings of Col wild-type (A) and *rld1 rld2 rld3 rld4* (*rld1; 2; 3; 4*). **C and D**. Embryos of Col (**C**) and *rld1; 2; 3; 4* (**D**). **E-H**. *DR5rev:GFP* (**E, F**) and PIN1-GFP (**G, H**) expression in WT-like (**E, G**) and *rld1; 2; 3; 4* (**F, H**) embryos dissected from ovules of plant homozygous for *rld1*, *rld3*, and *rld4* and heterozygous for *rld2* at the heart stage.

1-2 LZYs recruit RLDs to the plasma membrane

rld1 rld4 mutants exhibited a significant reduction in the level of GFP-tagged PIN3 (PIN3-GFP), which itself is a member of auxin efflux carrier PIN family expressed in root cap columella cells. Moreover, *rld1 rld2 rld3 rld4* quadruple

mutant embryos and seedlings displayed severe morphological defects (Figure 2A-D). In the quadruple mutant embryos, a severe reduction of PIN1-GFP and abnormal auxin distribution pattern were observed (Figure 2E-H). These embryonic phenotypes closely resembled those of *gnom*, a loss-of-function mutant of an ARF-GEF GNOM involved in the trafficking of PIN proteins. These findings suggest that the RLDs could regulate auxin flow in the same pathway as GNOM to control PIN proteins not only in root gravitropism, but also in plant development.

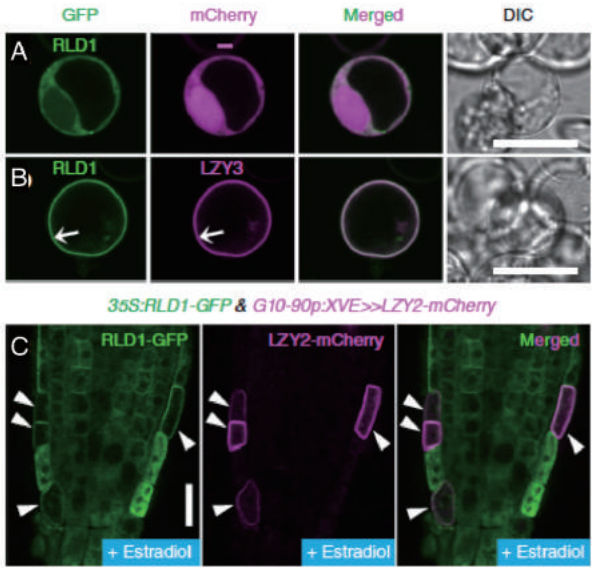

Figure 3. LZYs recruits RLDs to the plasma membrane. **A and B**. Co-expression of RLD1-GFP with mCherry (**A**) and LZY3-mCherry (**B**) in Arabidopsis protoplasts. **C**. Localization of RLD1-GFP (left) and LZY2-mCherry (middle) in the primary root harboring *35Sp:RLD1-GFP* and estradiol-inducible *LZY2-mCherry*. Arrowheads indicate LZY2-mCherry-expressing cells, where RLD1-GFP colocalized with LZY2-mCherry in the plasma membrane.

While RLD-GFPs were observed in the cytoplasm in Arabidopsis protoplast cells, they were localized on the plasma membrane only when LZY2/3-mCherry were co-expressed (Figure 3A, B). Chemical inducible expression of LZY2-mCherry in the root cells constitutively expressing RLD1-GFP led to the localization of RLD1-GFP on the plasma membrane whereas RLD1-GFP was detected in the cytosol in cells not expressing LZY2-mCherry (Figure 3C).

We also demonstrated through yeast two-hybrid experiments and *in vitro* pull-down assays that the CCL domain on the C-terminus of LZY and the BRX domain on the C-terminus of RLD are responsible for the interaction between LZY3 and RLDs. The interaction between the CCL domain and the BRX domain was required for their co-localization of LZY and RLD on the plasma membrane in protoplasts. To elucidate the structural basis of the interaction, we determined the crystal structures of the RLD2 BRX domain bound to the LZY3 CCL peptide at 1.35 Å resolution. The structural analysis reveals the mode of the interaction as an intermolecular β-sheet between the CCL and the BRX domain in addition to the structure of the BRX domain (Figure 4). Based on the structural analyses, we introduced mutations that impaired the interaction into the BRX or the CCL and demonstrated the importance of the interaction for gravitropism. These findings indicate RLDs, possible regulators of PIN trafficking, are recruited from the cytosol to the plasma membrane by LZYs through the interaction between the BRX domain and the CCL domain.

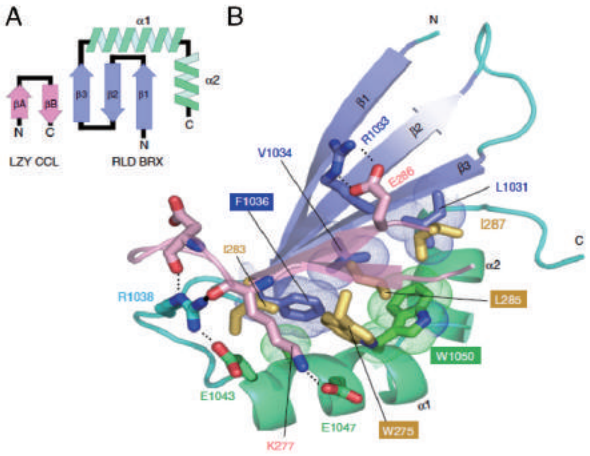

Figure 4. The structure of the CCL-BRX complex. **A**. Topology diagram of RLD2 BRX domain bound to LZY3 CCL. **B**. Ribbon representation of the crystal structure of the BRX-CCL complex. Color codes are as in **A**. The RLD2 BRX domain comprises a three-stranded antiparallel β-sheet (blue) and two α-helices (green). The LZY3 CCL peptide adopts a β-hairpin structure (pink). Dashed lines represent inter-molecular hydrogen bonds. The colors of corresponding positions in the CCL-BRX complex at which mutations were introduced in RLD1-GFP and LZY3-mCherry are reversed.

1-3 LZY3 controls RLD1 localization in columella cells of lateral roots

PIN3 is involved in the asymmetric auxin distribution in roots, and has been reported to polarly localize on the plasma membrane in the columella cells. Interestingly, we have found that LZY3-mCherry is also polarly localized on the basal side of the plasma membrane in the columella cells of lateral roots when plants are vertically grown. Intriguingly, 180˚ rotation of plants expressing LZY3-mCherry reduces the signal of LZY3-mCherry on the basal side of the plasma membrane and increases the signal on the apical side of plasma membrane where the latest gravity direction is at least 30 minutes after the rotation. This observation indicates that LZY3 is polarly localized on the plasma membrane in response to gravistimulation (Figure 5A). RLD1-GFP exhibits the same behavior as LZY3-mCherry in the columella cells whereas the asymmetric distribution of PIN3-GFP is observed 300 minutes after the rotation. Taken together, we have proposed a model in which gravity stimuli leads to polarization of LZYs and the polarized LZYs recruit RLDs on the plasma membrane in order to establish the polar auxin transport (Figure 5B). Little is known about how the amyloplast sedimentation information is transduced in the form of localization of LZYs and the molecular function of RLDs in gravity signaling. Accordingly, we are now exclusively focusing on revealing those mechanisms.

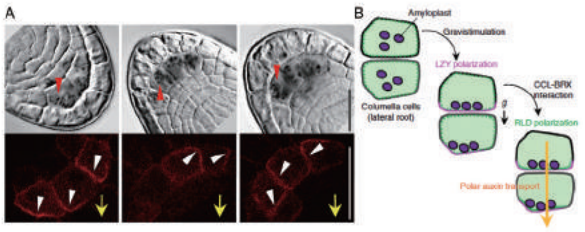

Figure 5. Asymmetric localization of LZY3-mCherry toward the direction of gravity and the model on gravity signaling. **A**. The localization of LZY3-mCherry in the lateral root tips of *lzy1 lzy2 lzy3* seedlings harboring *LZY3p:LZY3-mCherry* at the stage 2 before rotation (left) and at 5 min (middle), 30 min (right) after 180˚ rotation. White and red arrowheads indicate polarized LZY3-mCherry localization and amyloplasts, respectively. The yellow arrows indicate the direction of gravity estimated from the growth orientation of lateral root tips. **B**. Schematic diagrams of LZY-RLD-mediated gravity signaling in columella cells of lateral roots.

II. Determination mechanism of gravitropic setpoint angle

Plant posture is controlled by various environmental cues, such as light, temperature, and gravity. Their overall architecture is determined by the growth angles of lateral organs, such as roots and branches. The branch growth angle affected by gravity is known as the gravitropic setpoint angle (GSA). Many gravitropic mutants show abnormal GSA in lateral branches; meaning they produce wider growth angle phenotypes likely due to reduced gravitropism. Lateral branches of Arabidopsis *shoot gravitropism* (*sgr*) mutants lacking the endodermal cells, which themselves are shoot statocytes, grow horizontally as well as those possessed by wild type plants grown under microgravity condition.

The primary shoots of *lzy1 lzy2 lzy3* triple mutants exhibited non-responsiveness to gravistimulation, so it was expected that the lateral branches of the triple mutant would grow horizontally like *sgr* mutants do. However, they unexpectedly grew downward and showed positive gravitropism upon gravistimulation. Moreover, primary and lateral roots of *lzy2 lzy3 lzy4* triple mutant showed negative gravitropism. We accordingly refer to the phenotype of reversed growth direction observed in primary roots and lateral branches of *lzy* triple mutants as “anti-gravitropic”.

It has been proposed that GSA is determined by balancing two opposing growth components: gravitropism and anti-gravitropic offset (AGO). We assumed that the balance between gravitropism and AGO was disrupted in *lzy* triple mutants, and hypothesized that AGO would be manifested as an “anti-gravitropic” phenotype. The molecular mechanisms underlying gravitropism have been studied extensively, but little is known about the nature of AGO. To investigate the mechanism of GSA control, we are currently focusing on understanding the nature of AGO by analyzing “anti-gravitropic” phenotypes of *lzy* triple mutants.

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〔Original papers〕

- Furutani, M., Hirano, Y., Nishimura, T., Nakamura, M., Taniguchi, M., Suzuki, K., Oshida, R., Kondo, C., Sun, S., Kato, K., Fukao, Y., Hakoshima, T., and Morita, M.T. (2020). Polar recruitment of RLD by LAZY1-like protein during gravity signaling in root branch angle control. Nat. Commun. *11*, 76. doi: 10.1038/s41467-019-13729-7
- Kimata, Y., Kato, T., Higaki, T., Kurihara, D., Yamada, T., Segami, S., Morita, M.T., Maeshima, M., Hasezawa, S., Higashiyama, T., Tasaka, M., and Ueda, M. (2019). Polar vacuolar distribution is essential for accurate asymmetric division of *Arabidopsis* zygotes. Proc. Natl. Acad. Sci. USA *116*, 2338-2343. doi: 10.1073/pnas.1814160116
- Mergner, J., Frejno, M., List, M., Papacek, M., Chen, X., Chaudhary, A., Samaras, P., Richter, S., Shikata, H., Messerer, M., Lang, D., Altmann, S., Cyprys, P., Zolg, D.P., Mathieson, T., Bantscheff, M., Hazarika, R.R., Schmidt, T., Dawid, C., Dunkel, A., Hofmann, T., Sprunck, S., Falter-Braun, P., Johannes, F., Mayer, K.F.X., Jürgens, G., Wilhelm, M., Baumbach, J., Grill, E., Schneitz, K., Schwechheimer, C., and Kuster, B. (2020). Mass-spectrometry-based draft of the *Arabidopsis* proteome. Nature *579*, 409-414. doi: 10.1038/s41586-020-2094-2
- Ohbayashi, I., Huang, S., Fukaki, H., Song, X., Sun, S., Morita, M.T., Tasaka, M., Millar, A.H., and Furutani, M. (2019). Mitochondrial pyruvate dehydrogenase contributes to auxin-regulated organ development. Plant Physiol. *180*, 896-909. doi: 10.1104/pp.18.01460

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

- Shindo, M., Makigawa, S., Matsumoto, K., Iwata, T., Wasano, N., Kano, A., Morita, M.T., and Fujii, Y. Essential structural features of (2Z,4E)-5-phenylpenta-2,4-dienoic acid for inhibition of root gravitropism. Phytochemistry 2020 Feb 1. doi: 10.1016/j.phytochem.2020.112287

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- Nakamura, M., Nishimura, T., and Morita, M.T. (2019). Bridging the gap between amyloplasts and directional auxin transport in plant gravitropism. Curr. Opin. Plant Biol. *52*, 54-60. doi: 10.1016/j.pbi.2019.07.005
- Nakamura, M., Nishimura, T., and Morita, M.T. (2019). Gravity sensing and signal conversion in plant gravitropism. J. Exp. Bot. *70*, 3495-3506. doi: 10.1093/jxb/erz158

LABORATORY OF GENOME INFORMATICS

Assistant Professor
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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 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 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. Through 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 by 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 entirety of each ortholog table. For some closely related taxa, MBGD provides conserved synteny information calculated using the CoreAligner program. MBGD additionally provides a 'MyMBGD' mode, which allows users to add their own genomes to MBGD.

MBGD now contains 6318 genomes, including 5861 bacteria, 254 archaea, and 203 eukaryota. These data sets are classified based on the hierarchical ortholog classification strategy described in the section below.

II. Hierarchical strategy for creating ortholog tables

MBGD previously calculated all-against-all similarities among the stored genomes and independently created two types of ortholog tables: the standard ortholog table containing one representative genome from each genus covering the entire taxonomic range, and the taxon specific ortholog tables containing the genomes belonging to each taxonomic group (species, genus, family and so on). Although this approach enabled us to effectively calculate a set of ortholog tables for both closely related and distantly related genome comparisons, a considerable amount of information may be lost from the standard ortholog table, given that within-species and within-genus genomic diversity is generally large.

To address this problem, we developed a stepwise protocol to construct orthologous relationships. First, for each species with 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 with at least two species, all-against-all similarities among the species-level pan-genomes are calculated 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. Using this method, we can reduce the computation time required for all-against-all similarities.

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

As a core technology of our comparative genomics tools, we have developed a rapid automated method of ortholog grouping, named DomClust, which allows us to simultaneously compare numerous 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 when required. As a result, the procedure can split genes into the domains minimally required for ortholog grouping.

We have 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. On the basis of this idea, we have developed a refinement pipeline to improve domain-level clustering, DomRefine, by optimizing DSP scores. 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 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 occur.

IV. Development and application of a workbench for comparative genomics and transcriptomics

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. By combining these basic operations, various comparative analyses can be performed. 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. Among these, we applied RECOG to the comparative analyses of transcriptomic data of *Chattonella antiqua* and other harmful algae causing red tide in collaboration with Dr. Shikata (FRA). In this analysis, we compared two newly determined and three existing RNA-seq datasets of harmful algae and 10 existing genome sequences of various algae and a plant model organism, *Arabidopsis thaliana* using RECOG, and identified common sequence features among orthologous genes belonging to harmful algae that may be related to red tide outbreaks and their toxicity to fish.

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

Orthology is a key to integrating knowledge of various organisms through comparative analysis. In order to integrate genomic data and various types of biological information with this idea, we have constructed an ortholog database using Semantic Web technology. 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). OrthO is now superseded by the Orthology Ontology (ORTH) that integrates OrthO and other ontologies and is adopted by other orthology resources.

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 are developing a method (FindIsland) to identify a set of non-core genes that have conserved gene order by using a modified version of the CoreAligner algorithm (Figure 1). We applied the method to the sets of genomes of prokaryotic species stored in MBGD and found that in many cases the resulting conserved clusters correspond to known mobile genetic elements and/or have sequence features common to known genomic islands.

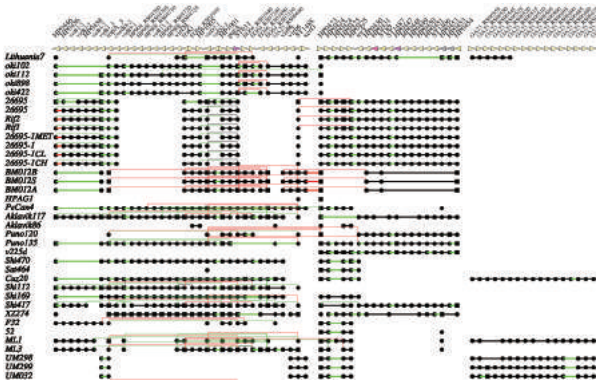

Figure 1. Visualization of the genomic islands identified by the FindIsland program.-

Publication List:

〔Original papers〕

- Matsunami, M., Suzuki, M., Haramoto, Y., Fukui, A., Inoue, T., Yamaguchi, K., Uchiyama, I., Mori, K., Tashiro, K., Ito, Y., Takeuchi, T., Suzuki, K.-I.T., Agata, K., Shigenobu, S., and Hayashi, T. (2019). A comprehensive reference transcriptome resource for the Iberian ribbed newt *Pleurodeles waltl*, an emerging model for developmental and regeneration biology. DNA Res. *26*, 217-229. doi: 10.1093/dnares/dsz003
- Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., and Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. Nat. Plants *5*, 890-901. doi: 10.1038/s41477-019-0477-x
- Shikata, T., Takahashi, F., Nishide, H., Shigenobu, S., Kamei, Y., Sakamoto, S., Yuasa, K., Nishiyama, Y., Yamasaki, Y., and Uchiyama, I. (2019). RNA-Seq analysis reveals genes related to photoreception, nutrient uptake, and toxicity in a noxious red-tide raphidophyte *Chattonella antiqua*. Front. Microbiol. *10*, 1764. doi: 10.3389/fmicb.2019.01764
- Uchiyama, I., Mihara, M., Nishide, H., Chiba, H., and Kato, M. (2019). MBGD update 2018: Microbial genome database based on hierarchical orthology relations covering closely related and distantly related comparisons. Nucleic Acids Res. *47*, D382-D389. doi: 10.1093/nar/gky1054

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

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NONAKA, Shigenori

NIBB Research Fellow: *YOKE, Hiroshi*
Postdoctoral Fellow: *TANIGUCHI, Atsushi*
Visiting Scientist: *KONDO, Akiko*
Technical Assistant: *ISHIBASHI, Tomoko*

Our laboratory is currently pursuing two paths of scientific inquiry that are of interest to developmental biology. One 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, 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.

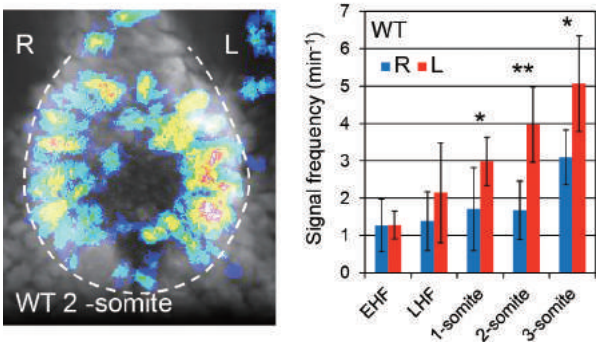

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

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 due to 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 cell migration in zebrafish embryos, cleared mouse brains, etc.

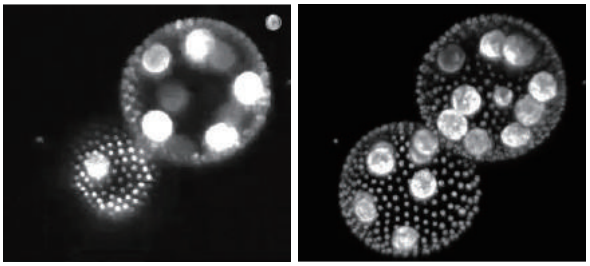

Figure 2. Images of floating volvox taken by ezDSLM with ETL. Left: Single optical section. Right: Maximum intensity projection.

Publication List:

〔Original papers〕

- Kaji, T., Song, C., Murata, K., Nonaka, S., Ogawa, K., Kondo, Y., Ohtsuka, S., and Palmer A.R. (2019). Evolutionary transformation of mouthparts from particle-feeding to piercing carnivory in Viper copepods: Review and 3D analyses of a key innovation using advanced imaging techniques. Front. Zool. *16*, 35. doi: 10.1186/s12983-019-0308-y
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- Yoon, Y., Park, J., Taniguchi, A., Kohsaka, H., Nakae, K., Nonaka, S., Ishii, S., and Nose, A. (2019). System level analysis of motor-related neural activities in larval *Drosophila*. J. Neurogenet. *33*, 179-189. doi: 10.1080/01677063.2019.1605365

LABORATORY FOR BIOTHERMOLOGY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

NIBB Research Fellow: *SAKAMOTO, Joe*
CREST Researcher: *KAMIKAWA, Yuko*
JSPS Postdoctral Fellow: *SUZUKI, Miyuki*
Technical Assistant: *KINOSHITA, Chie*
TAMADA, Tomoko

Temperature is an important parameter for living organisms. Cell activity is affected by temperature since the reaction ratio and stability of molecules in cells depends on it. Environmental temperatures which organisms function in are variable, and the biological system that regulates body temperature, homeostasis, differs among various organisms. Furthermore, temperature in cells or at the molecular level has not been widely discussed to date. Nano-scale thermometers utilizing fluorescent proteins and dyes have been developed by numerous research groups including ours, and heterogeneity of temperatures in the micro environments of living cells have been reported on by said groups (Okabe *et al*, Nat. Commun. 2014, Kiyonaka *et al*, Nat. Methods 2014, and Nakano *et al*, PLoS One 2017). However, the meaning and mechanisms in single cells remain unclear. Thus, our group is embarking upon a new research field, biothermology, through the investigation of the nature of temperature in living organisms ranging from nano to macro.

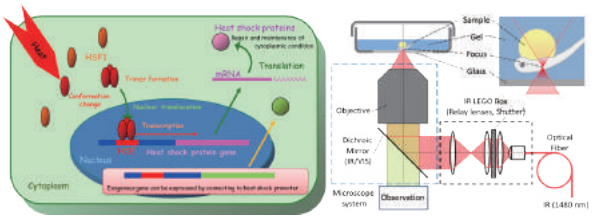

Figure 1. Schematic illustration of heat shock response of cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

Our research group employs various and original biothermological research technologies . One of these is the infrared (IR) laser application, which enables single-cell or subcellular local heating by focusing IR through microscope objective. This technology was originally developed for laser induced gene expression in the targeting of single-cells through the heat shock response, called IR-LEGO; InfraRed Laser Evoked Gene Operator (Kamei *et al*, Nat. Methods 2009) (Figure 1). This local heating technique can be used to analyze the thermodynamics of a cell via the use of a nano-scale thermometer;the second of our abovementioned original techniques. To analyze temperature distribution at subcellular resolutions, a nano-scale thermometer is required. For this reason, we have focused on fluorescent proteins, and developed a genetically encoded thermometer, the so called gTEMP, with Dr. Takeharu Nagai from Osaka University (Nakano *et al*, PLoS One 2017). This thermometer has some unique properties such as rapid response and applicability to wide temperature range. By utilizing this thermometer, we are developing a high-speed thermal imaging microscope system combined with IR irradiation optics. Through the analysis of thermal dynamics in cells, we are trying to reveal how temperature heterogeneity is generated within a single cell and to consider its meaning from a biological standpoint.

Furthermore, our group is trying to improve the IR-LEGO technique itself. We initially applied this system to many organisms including animals and plants, such as medaka, nematode, flies, frogs, and *Araidopsis*, moss, in collaborative research projects in cooperation with other laboratories. Because the heat shock response (HSR) is a transient response, gene expressions after IR-LEGO operation are also transient. Despite this, some of our collaborators wanted to realize cell fate mapping, which requires a long-term gene expression after IR irradiation. Accordingly, we employed Cre/LoxP recombination system. As shown in Figure 2, we established a cre driver line, which possesses a heat shock promoter and loxP effector line which can permanently label irradiated cell lineages by fluorescent proteins. By using these transgenic lines, evidence was provided that indicated that exoskeletal tissues in trunk region came from the mesoderm (Shimada *et al*, Nat. Commun. 2013).

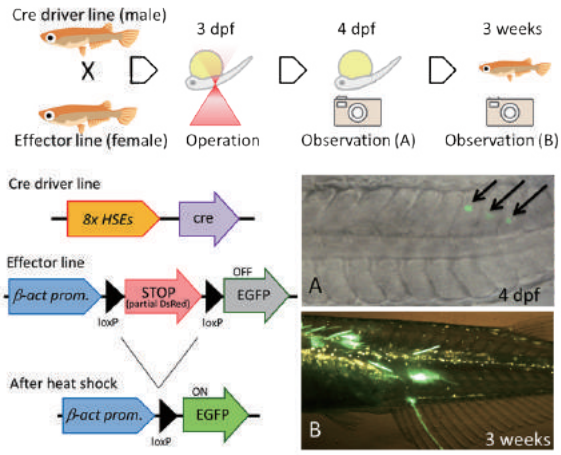

Figure 2. Long-term gene expression with cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

The HSR is a conservative stress response system found in almost all organisms. In contrast, organisms live in various temperatures, and HSR essentially is the upper limit of habitable temperature range for each organism. This means that the preset temperature of HSR may differ among organisms. A key factor of this limitation is heat shock factor 1 (HSF1); a homologue within each organism. By substituting HSF1, it may be possible to control the preset temperature of HSR, and this substitution could be applied to the improvement of HSR efficiency in IR-LEGO technology. We are now trying to lower the HSR temperature of medaka by substituting Japanese flounder HSF1. This HSF1 substitution project provides us with various significant information and evidence related to molecular evolution and adaptation to environmental temperature. Therefore, this project also contributes to the study of biothermology.

We also promote other collaborative studies using microscopic techniques. One is a "clear observation project" for deep seeing into living organisms using adaptive optics (AO), which have been thoroughly developed in the field of astronomy as a key technology found in large telescopes, such as the Subaru telescope in Hawaii. Although observation using Earth based telescopes may be interfered with due to fluctuations in the atmosphere, AO can mitigate this. However, living materials have particular refractive indexes, so some organelles may hinder the ideal optical path for microscope observation, which is similar to the situation that exists regarding the atmosphere and telescopes. AO can also compensate for this disturbance by sensing and correcting wave fronts by using a wave front sensor and deformable mirror. Hence, we have developed a custom-made wide-field microscope equipped with an AO system for the observation of living organisms in collaboration with Dr. Yosuke Tamada from Utsunomiya University and Dr. Yutaka Hayano from the National Astronomical Observatory of Japan (NAOJ). By utilizing this microscopic system, we have successfully acquired high-resolution bright field and fluorescent images of living cells. Furthermore, AO can also be applied to IR-LEGO technology to improve IR energy focusing of irradiation in a manner similar to improving the sight of living organisms.

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- Nakayama, T., Shimmura, T., Shinomiya, A., Okimura, K., Takehana, Y., Furukawa, Y., Shimo, T., Senga, T., Nakatsukasa, M., Nishimura, T., Tanaka, M., Okubo, K., Kamei, Y., Naruse, K., and Yoshimura, T. (2019). Seasonal regulation of the lncRNA LDAIR modulates self-protective behaviours during the breeding season. Nat. Ecol. Evol. *3*, 845-852. doi: 10.1038/s41559-019-0866-6
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- Abe, G., Hayashi, T., Yoshida, K., Yoshida, T., Kudoh, H., Sakamoto, J., Konishi, A., Kamei, Y., Takeuchi, T., Tamura, K., and Yokoyama, H. (2020). Insights regarding skin regeneration in non-amniote vertebrates: Skin regeneration without scar formation and potential step-up to a higher level of regeneration. Semin. Cell Dev. Biol. *100*, 109-121. doi: 10.1016/j.semcdb.2019.11.014
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LABORATORY OF NUCLEAR DYNAMICS†

Specially Appointed Associate Professor
MIYANARI, Yusuke

JSPS Postdoctoral Fellow: *KURIHARA, Misuzu*
Research Staff: *TAGAWA, Ayako*
Technical Assistant: *SANBO, Chiaki*
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.

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.

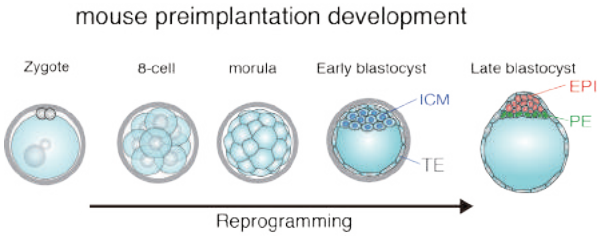

Figure 1. Lineage allocation in mouse preimplantation development

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.

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.

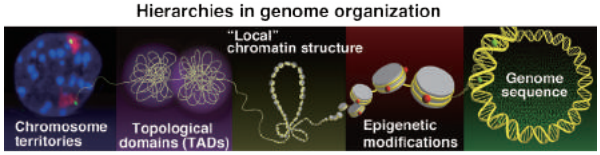

Figure 2. Hierarchical chromatin structure

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.

†: This laboratory was closed on 31 March, 2019.

LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY†

Specially Appointed Associate Professor
KAWADE, Kensuke

Visiting Graduate Student: TOMOI, Takumi
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.

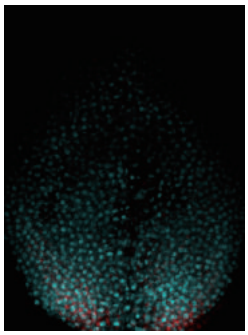

Figure 2. A merged image of the leaf primordia (around 200-μm length) expressing genetically engineered mobile or immobile AN3 (cyan or red, respectively). The distribution of the mobile AN3 gradually spreads along the leaf proximal-to-distal axis (from down to top), which is broader than that of the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

Publication List:

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

● Tomoi, T., Kawade, K., Kitagawa, M., Sakata, Y., Tsukaya, H., and Fujita, T. Quantitative imaging reveals distinct contributions of SnRK2 and ABI3 in plasmodesmatal permeability in *Physcomitrella patens*. Plant Cell Physiol. 2020 Feb. 26. doi: 10.1093/pcp/pcaa021

†: This laboratory was closed on 31 March, 2019.

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

Professor
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
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 renovated the 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 2019, approximately 100 researchers came to use our facility and developed active collaborations, which consequently resulted in 21 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 HiSeq, NextSeq and MiSeq (Illumina), 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 2019, we carried out 57 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.

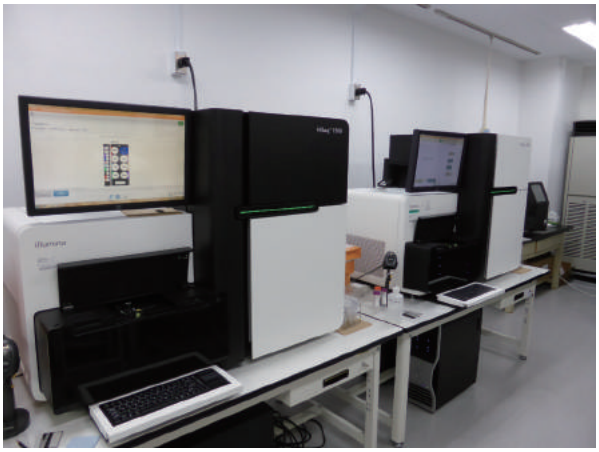

Figure 1. Next-generation sequencer

Proteomics

Two types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2019, 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)

Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

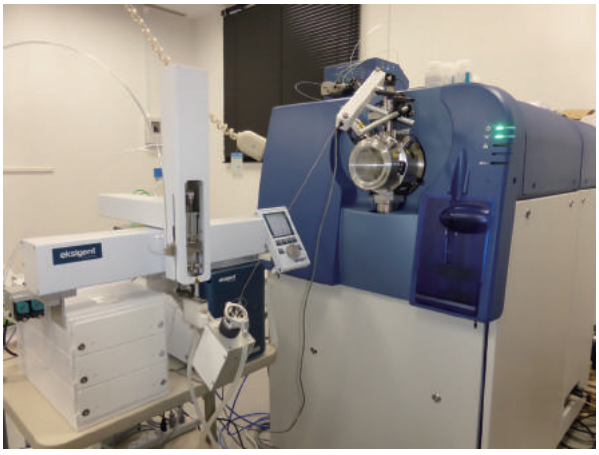

Figure 2. Triple TOF LC/MS/MS System

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)

Research activity by S. Shigenobu

Professor Shuji Shigenobu is the principal investigator of the Laboratory of Evolutionary Genomics. Refer to the laboratory page for details.

Publication List on Cooperation:

〔Original papers〕

- Bessho-Uehara, M., Yamamoto, N., Shigenobu, S., Mori, H., Kuwata, K., and Oba, Y. (2020). Kleptoprotein bioluminescence: Parapriacanthus fish obtain luciferase from ostracod prey. Sci. Adv. *6*, eaax4942. doi: 10.1126/sciadv.aax4942
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SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

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

The Spectrography and Bioimaging Facility assists both 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 (please 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.

Standard Instruments: Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum ranging 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.

In addition to the other action spectroscopical studies concerning various regulatory and damaging effects of light on living organisms, research involving biological molecules and artificial organic molecules have been conducted since it was set up. 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 members of NIBB.

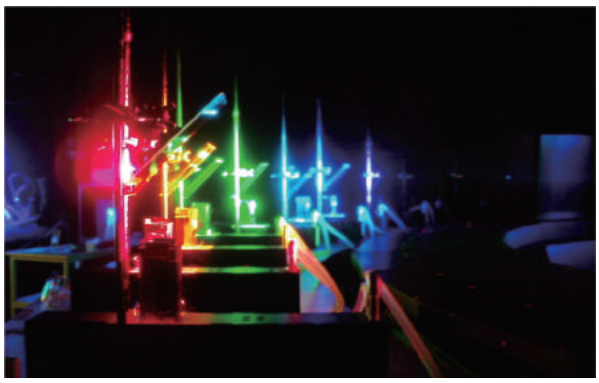

Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously to samples stored in cooling chambers.

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. Starting from 2016, we commenced two new types of Collaborative Research Programs. One is a new category within 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 the report submitted by Dr. Shigenori Nonaka's Laboratory for Spatiotemporal Regulations). Dr. 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

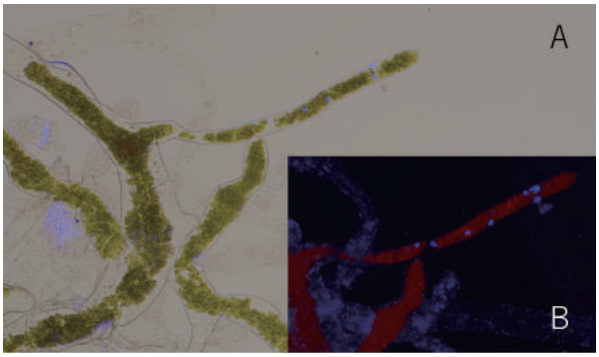

Figure 2. Microscope images of green algae, *Codium fragile*. Sample was stained by hoechst33342. (A) Bright and blue fluorescent marge image. (B) Blue fluorescent and red fluorescent marge image. Images were taken by visitor of cooperation program from Osaka City University (Seki, Soichiro). *C. fragile* (KU-0654) was provided by KU-MACC, Kobe University.

(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 aimed at animals and plants.

Workshop, Symposium and Training course

In 2019, we held the 7th biological image processing training course in cooperation with Drs. Kagayaki Kato, Shigenori Nonaka, Takashi Murata and Hiroshi Koyama. We also have been holding a "Bioimaging Forum" every year, which discusses bioimaging from various technical perspectives such as microscopy, new photo-technology, and computer science. This year we planned on staging the 14th NIBB Bioimaging Forum focused on micro CT technology in February, 2020. However, this forum was postponed until next year due to the global COVID-19 pandemic.

Research activity by Y. Kamei

Specially Appointed Associate Professor Yasuhiro Kamei is the principal investigator of Laboratory for Biothermology. For details, please refer to the laboratory page.

Publication List on Cooperation

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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. To achieve this, the facility maintains high-performance computers with large-capacity storage systems. It accordingly supports the development of data analysis pipelines and database construction based on these systems, and also sets up websites 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 efficiently processed. Some personal computers and color printers are also available for use. 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 provided support in the construction and maintenance of published databases of various model and non-model organisms in particular. These include XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), iNewt (*Pleurodales waltl*), The Plant Organelles Database, MBGD (microbial genomes), and DB-HABs (harmful algal blooms).

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, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (http://www.nibb.ac.jp/en).

Figure 1. Biological Information Analysis System

Research activity by I. Uchiyama

Assistant Professor Ikuo Uchiyama is the principal investigator of the Laboratory of Genome Informatics. For more 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, which have markers placed on them, 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
	KITAZUMI, Noriaki

The worldwide genome project has almost been completed aThe worldwide genome project has almost been completed and basic biological research is now in a post-genome era in which researchers focus 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 was also opened at the end of 2003 in the Yamate area of NIBB.

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) was opened in the Myodaiji area of NIBB. Since then, 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.

From January 1, 2019 to March 31, 2020, 4,348 mice (13 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 61,200 mice (including pups bred in the facility) were taken out.

Figure 1. Mouse breeding room in the Yamate area

Figure 2. Large sized autoclave in the Yamate area

A number of strains of genetically altered mice from outside the facility were brought into this area after microbiological cleaning using *in vitro* fertilization-embryo transfer techniques (9 transgenic lines), and stored using cryopreservation (41 transgenic lines). The frozen eggs of 32 mice lines were taken out of the facility.

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

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 these embryos under optimal conditions, thus removing biohazard risks.

For researchers who employ fish as an experimental model, 480 tanks (1 liter) and 450 tanks (3 liter) are available for medaka and zebrafish, respectively. Additionally, water can be maintained to suit the conditions desired for fish breeding. A medaka line that allows gene induction by heat treatment, in combination with a cre/loxP system, has been developed using this facility. All the rooms are qualified to meet the criteria for transgenic animals, allowing researchers to generate and maintain these important biological tools.

Figure 4. Liquid nitrogen tank

In 2019, 0 zebrafish (0 fertilized eggs) were brought to the facility nor were there any fertilized eggs or chicken embryos brought in or taken from the laboratory. The animals housed within the facility were used for research activities in neurobiology and developmental biology.

III. Research activities

The associate professor of this center, Dr. Eiji Watanabe, is the principal investigator of the Laboratory of Neurophysiology, which studies various mechanisms of the visual system using a psychophysical approach. For more details, please refer to the laboratory's page.

MODEL PLANT RESEARCH FACILITY

● ***Plant Culture Laboratory***

Assistant Professor:	*HOSHINO, Atsushi*
	TSUGANE, Kazuo
Technical Staff:	*MOROOKA, Naoki*
Technical Assistant:	*YAMAGUCHI, Chinami*

The Plant Culture Laboratory manages facilities for the cultivation of plants in general and 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, such as 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 up all year long 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, the castor bean, 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 and several aquatic ferns. 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 and the common grass yellow butterfly.

Between January 2019 and March 2020, 10 culture boxes were replaced and newer ones were introduced in their place.

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 laboratory's page.

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 main 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 science 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 zebrafish bioresources.

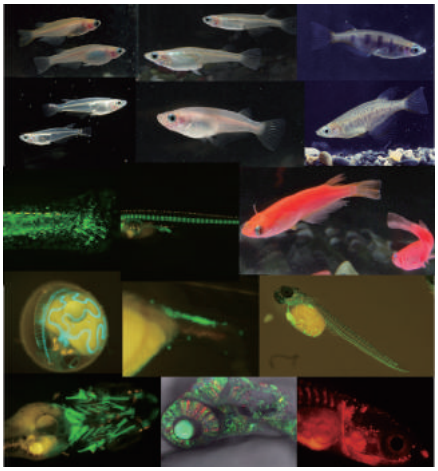
I. NBRP Medaka (*Oryzias latipes*)

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.

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.

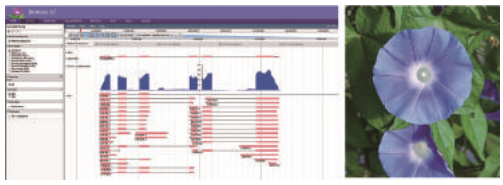


Medaka resources provided from NBRP medaka

II. NBRP Morning Glory (*Ipomoea nil*)

Project Manager: HOSHINO, Atsushi

The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, that is studied worldwide, especially in the fields of 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 at Kyushu University. From January 2019 to March 2020, we have collected several mutant lines and DNA clones, and provided 21 mutant lines and 64 DNA clones to both local and international biologists.

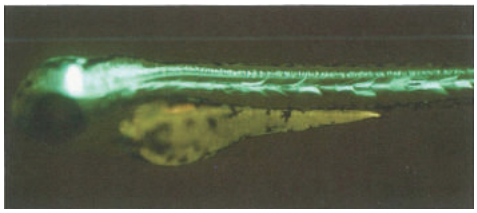


Left: The genome database (http://viewer.shigen.info/asagao/) contains the whole genome sequence, the transcriptome sequences, and the end sequences of the EST and BAC clones. The genome and mutant databases are linked by the sequence information of the mutations. Right: The flower phenotype of a mutant line.

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.

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

Head and Specially Appointed Professor
NARUSE, Kiyoshi

Research Staff: *AKIMOTO-KATO, Ai*
TANAKA, Ayako
Technical Assistant: *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.

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

The IBBP Center includes:

- earthquake proof structures capable of withstanding even very large scale quakes 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 2019, the IBBP Center stored 5,818 384-well and 112 96-well plates consisting of 2,244,864 clones as cDNA/BAC clones, 16,973 tubes for animal cells, plant and

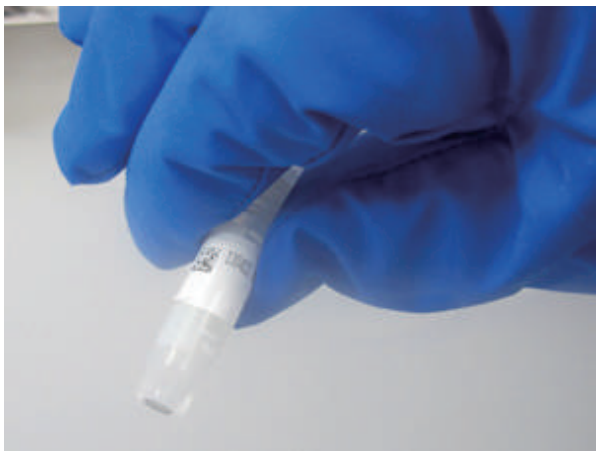


Figure 3. Cryo tube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

animal samples, proteins, genes and microorganisms, 6,174 133mm-straw tubes for sperm and 728 seed samples. In total 2,110,464 samples were stored.

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 2019, we have conducted 12 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 2019 on November 18-19, 2019 at the Tsukuba Center for Institutes, Tsukuba, Ibaraki, Japan. We had over 100 participants from several fields covering physics, chemistry, biology, and technology. We invited Prof. Tanaka at University of Tokyo as a plenary lecturer. Prof. Tanaka gave us a talk on the theoretical and structural view of water vitrification and crystallization processes.

Figure 4. Group photo of Cryopreservation conference 2019

Publication List on Cooperation:

〔Original paper〕

● Kaneko, T., and Nakagawa, Y. (2020). Genome editing of rodents by electroporation of CRISPR/Cas9 into frozen-warmed pronuclear-stage embryos. Cryobiology *92*, 231-234. doi: 10.1016/j.cryobiol.2020.01.016

CENTER FOR THE DEVELOPMENT OF NEW MODEL ORGANISMS

Head
UENO, Naoto

Professor
SHIGENOBU, Shuji

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 a limited number of species known as model organisms, which are easy to handle in a laboratory environment. However, this development has most likely left many interesting biological phenomena unexamined as their distinctive characteristics are observed only in a particular group of species. How we overcome this is an important challenge for biology hereafter.

To solve these problems, we must choose a species most suitable to analyze the phenomenon to be researched, and then establish it as a new model organism by developing methods using procedures that are necessary for modern biological analyses. 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. Through its activities, organisms that have been out of reach of scientific research were designated as new model organisms. For example, we study aphids and sea anemones to understand the symbioses, and rhinoceros beetles to get insights into the sexual dimorphism. We are refining various techniques for studying targeted new model organisms ranging from genome analysis to genetic engineering to build seamless workflows that will be shared among the research community.

Research activity by S. Shigenobu

Professor Shuji Shigenobu is the principal investigator of the Laboratory of Evolutionary Genomics. Refer to the laboratory page for details.

Research activity by K. Suzuki

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

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.

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 the phenomenon 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 issue, 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).

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 tech-

niques. *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 researching the molecular basis of organ regeneration using this newt. In addition, we widely support researchers who attempt to develop new model organisms contributing to the up-coming biology.

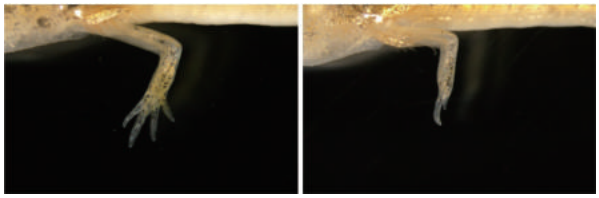


Figure 2. A limb-specific enhancer (ZRS/MFCS1) of *sonic hedgehog* crispant in *P. waltl*. Phenotypes of limb regeneration in wild and ZRS/MFCS1 crispant (left and right, respectively). Unlike in normal limb regeneration in the wild type, severe reduction of digit formation was seen in ZRS/MFCS1 crispant.

Publication List:

〔Original papers〕

- Matsunami, M., Suzuki, M., Haramoto, Y., Fukui, A., Inoue, T., Yamaguchi, K., Uchiyama, I., Mori, K., Tashiro, K., Ito, Y., Takeuchi, T., Suzuki, K.-I.T., Agata, K., Shigenobu, S., and Hayashi, T. (2019). A comprehensive reference transcriptome resource for the Iberian ribbed newt *Pleurodeles waltl*, an emerging model for developmental and regeneration biology. DNA Res. *26*, 217-229. doi: 10.1093/dnares/dsz003
- Yoshinouchi, Y., Shimizu, S., Lee, J.S., Hirano, M., Suzuki, K., Kim, E.Y., and Iwata, H. (2019). In vitro assessment of effects of persistent organic pollutants on the transactivation of estrogen receptor α and β (ERα and ERβ) from the Baikal seal (*Pusa sibirica*). Ecotox. Environ. Safe. *181*, 463-471. doi: 10.1016/j.ecoenv.2019.06.033

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor
KODAMA, Ryuji

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

Technical Assistant:
HAYASHI, Tomoko

The Center for Radioisotope Facilities (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 keeping with this, the CRF's technical and support staff maintain controlled areas in compliance with the relevant laws.

The following is an outline of the notable activities that CRF conducted in 2019.

1. Revision of CRF's local rules

We revised the rules as they pertain to our local facilities (referred to as "Radiation Damage Protection Rules") in the Myodaiji and Yamate areas to conform with amendments to national laws concerning radioisotope protection. Major revisions that were included are as follows:

1) Preparation of the subordinate rules

We prepared the following manuals of which are deemed subordinate to the local rules mentioned above: a) users' guide b) management and inspection manual c) emergency response manual d) emergency information provision manual

2) Specification of the manager and responsible party for each action described in the rules

3) Revision of the radiation safety education and training protocols

We decided the necessary hours, standards for partial omissions, and protocols to change the necessary hours for radiation safety education and training.

2. Changes regarding usable nuclides at Myodaiji area

Since there has been no practical use for gamma ray nuclides at the Myodaiji area for several years, we have decided to discontinue their use. Hence, the revised list of usable nuclides at this facility was approved by the Nuclear Regulation Authority in November 2019 (Table 1). We then subsequently stopped the operation of the gamma ray monitors and measuring instruments.

The number of registrants and the number of users at our facility from January 2019 to March 2020 are shown in Table 2. Users and visitors counted by the access control system in the controlled areas numbered 1,445 during this period. The numbers for each area are shown in Table 3. The annual changes concerning registrants and the number of totals per fiscal year are shown in Figure 1. The balance of radioisotopes received and used at the CRF is shown in Table 4. The figures for training courses on radioisotope handling are provided in Table 5.

Nuclides	State	Chemical form	Maximum permissible quantity		
			year	3 months	day
^{3}H	liquid	all	20GBq	20GBq	1GBq
^{14}C	liquid	all	5GBq	5GBq	500MBq
^{22}Na	Deleted				
^{32}P	liquid	all	4.5GBq	4.5GBq	500MBq
^{33}P	liquid	all	15GBq	15GBq	500MBq
^{35}S	liquid	all	15GBq	15GBq	500MBq
^{36}Cl	liquid	all	150MBq	150MBq	50MBq
^{42}K	Deleted				
^{45}Ca	liquid	all	2GBq	2GBq	200MBq
^{125}I	Deleted				

Table 1. Changes of usable nuclides at Myodaiji area

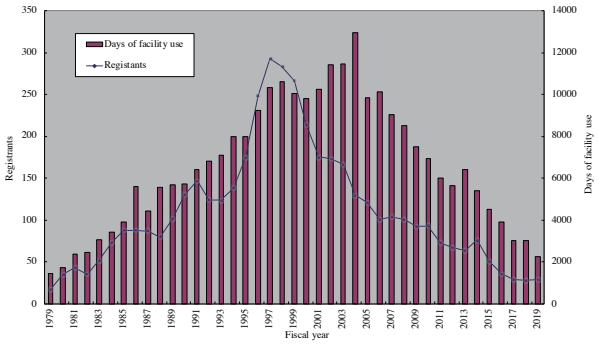


Figure 1. Changes of registrants and days of facility use per fiscal year

	Myodaiji Area	Yamate Area
Registrants	37	30
Users	17	21

Table 2. Numbers of registrants and users at the Myodaiji and Yamate areas from 2019 January to 2020 March

	Myodaiji Area	Yamate Area	Total
Users	641	460	1,101
Visitors	161	183	344
Total	802	643	1,445

Table 3. Users and visitors who entered each controlled area from 2019 January to 2020 March

		Myodaiji Area	Yamate Area	Total
^{125}I	Received	0	0	0
^{125}I	Used	0	0	0
^{35}S	Received	0	0	0
^{35}S	Used	0	0	0
^{32}P	Received	27,750	20,350	48,100
^{32}P	Used	22,940	29,600	52,540
^{14}C	Received	0	0	0
^{14}C	Used	0	0	0
^{3}H	Received	9,250	0	9,250
^{3}H	Used	0	0	0

Table 4. Balance of radioisotopes received and used (kBq) at each controlled area from 2019 January to 2020 March

training course	place	numbers of participant
Introductory course for beginners	Myodaiji	0
Introductory course for beginners	Yamate	0
Introductory course for experts	Myodaiji	2
Introductory course for experts	Yamate	1
Users training course	Myodaiji	33
Users training course	Yamate	19

*including English course

Table 5. Training courses for radiation workers in from 2019 January to 2020 March

RESEARCH ENHANCEMENT STRATEGY OFFICE

Director
UENO, Naoto

Deputy Director
HASEBE, Mitsuyasu

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: *BAN, Misato*
HOSHINO, Maki
UCHIMURA, Ai
OTA, Kyoko
Group Adviser: *FUJIMORI, Toshihiko*

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

The group's main activities in 2019

1) Press releases

The group issues news items concerning 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 brochures introducing NIBB. Design and distribution of posters of NIBB events.

4) Production of Videos

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

5) Organization of scientific outreach programs

Organization of the Summer Program for university students, and coordination of special classes for middle school students. Organization of the 27th NINS symposium for the public. Coordination of NIBB open house 2019 (p. 111).

INTERNATIONAL COOPERATION GROUP

Specially Appointed Assistant Professor (URA):	*TATEMATSU, Kiyoshi*
Technical Assistant:	*TAKAHASHI, Ritsue*
	COWAN, Glen
Group Advisor:	*UENO, Naoto*

Two of NIBB's missions is to continually explore the leading-edge of biology and to form research communities that link Japan to the world. To achieve this, it conducts scientific meetings such as the "NIBB Conference", as well as educational programs such as the "NIBB International Practical Course".

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), and has also signed a new cooperative agreement with the Center for Organismal Studies (COS) Heidelberg (Germany) in 2019.

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 also conducts the "NIBB International Collaborative Research Initiative" to promote high-level international collaborations between faculty members and researchers from around the world. It also invites researchers, who are at forefront in research fields, 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 NIBB from the institutes mentioned above. This group also supports NIBB internship students visiting from foreign countries, and the dispatch of SOKENDAI graduate students 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 engaged in by this group from Jan. 2019 to March 2020

1) Coordination of international conferences and the International Practical Course

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

- The 66th NIBB Conference/ABiS International Symposium "Cutting Edge Techniques of Bioimaging" Okazaki, Japan, February 17-18, 2019 (p. 100)
- The 67th NIBB Conference "Quest for Orthologs" Okazaki, Japan, July 31 – August 2, 2019 (p. 101)
- The 2nd NIBB-Princeton Symposium "Imaging and Quantitative Biology" Okazaki, Japan, October 28–30, 2019 (p. 102)

2) Support of visiting researchers to NIBB

This group supported visits of foreign researchers related to the following events (p. 103):

- One professor and one manager of Corporate Engagement Team coming from Princeton University (January and April, 2019)
- Managing Director and two junior professors coming from COS Heidelberg (May and September, 2019)
- Director coming from EMBL-EBI (August, 2019)

3) Support of education-related programs

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

- NIBB Internship Program 2019 (p. 110)

COLLABORATIVE RESEARCH GROUP

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

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 2019, this group hosted a total of 115 collaborative projects. The success of these collaborations is best illustrated by the 36 research papers that were jointly published during this year thanks to a number of these projects. One of the more remarkable achievements produced by this group was a transcriptome analysis of a harmful red tide alga *Chattonella antiqua*. This analysis was conducted by Dr. Tomoyuki Shikata (FEIS, FRA) and his colleagues in collaboration with the members of the NIBB core research facilities, Ikuo Uchiyama, Shuji Shigenobu and Yasuhiro Kamei. In this project, the abovementioned researchers conducted RNA-seq analysis of *C. antiqua* and identified several genes with functions possibly related to the generation and dynamics of red tide, such as photosynthesis, photoreception, nutrient uptake and superoxide production. The identified genes were further characterized by comparative analysis with orthologs in other harmful and non-harmful algae. The obtained data and analysis results are open to the public

through the website HAB-DB (http://hab.nibb.ac.jp/) in order to provide a basis for the understanding of the mechanism behind the development of red tide.

● Shikata, T., Takahashi, F., Nishide, H., Shigenobu, S., Kamei, Y., Sakamoto, S., Yuasa, K., Nishiyama, Y., Yamasaki, Y., and Uchiyama, I. (2019). RNA-Seq analysis reveals genes related to photoreception, nutrient uptake, and toxicity in a noxious red-tide raphidophyte *Chattonella antiqua*. Front. Microbiol. *10*, 1764. doi: 10.3389/fmicb.2019.01764

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 complement 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 advance 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. The room has been particularly useful for those wishing to hold meetings with small children by their side 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.

In 2019, the room had 18 registered users and also accommodated visitors attending meetings held in Okazaki. To create a safer environment, a surveillance camera and an air purifier were also installed.

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
NISHIMOTO, Yuki
SUGIURA, Hiroki
echnical Assistant: *ICHIKAWA, Chiaki*
ICHIKAWA, Mariko
OKA, Naomi
KOTANI, Keiko
SHIBATA, Emiko

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

● ***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*
Technical Staff: *BINO, Takahiro*

● ***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
KOTANI, Keiko

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.

The 66th NIBB Conference / ABiS International Symposium "Cutting Edge Techniques of Bioimaging"

Organizers: Naoto Ueno (NIBB), Toshihiko Fujimori (NIBB), Junichi Nabekura (NIPS), Masanobu Kano (NIPS / The University of Tokyo)

February 17 (Sun)–18 (Mon), 2019

Imaging technology has been widely applied to areas ranging from molecules, cells, tissues and all the way up to individual organisms. It has consequently become an essential analytical tool in the field of life science research. The development of new probes, microscopes and quantitative analytical methods, as well as the ability of being able to extract biological information from images, has progressed at a remarkable pace. As a result, we can now observe both multiple objects and the spatial temporal dynamics of the subjects simultaneously at both high speed and resolution.

NIBB has taken on a major role within the field of bioimaging in Japan as evidenced by its involvement in the advancement of various activities. These roles have involved the development of new observation technology and bioimage analysis methods and their circulation through bioimaging training courses, conducting the NIBB collaborative research projects for integrative bioimaging, and taking on the role of core institute in coordinating the Advanced Bioimaging Support (ABiS) activities in conjunction with NIPS. Against this backdrop, the 66th NIBB Conference, entitled "Cutting Edge Techniques of Bioimaging", was held in cooperation with ABiS at the Okazaki Conference Center from February 17th to 18th.

This conference aimed to deepen discussion concerning cutting edge bioimaging as well as sharing information regarding this area. In particular, there was a focus on super high resolution and live cell imaging. Events included:

- Lectures conducted by both domestic and international researchers engaged in cutting edge projects in the above-mentioned fields
- Imaging related poster presentations
- Short talks about various posters chosen from those on display

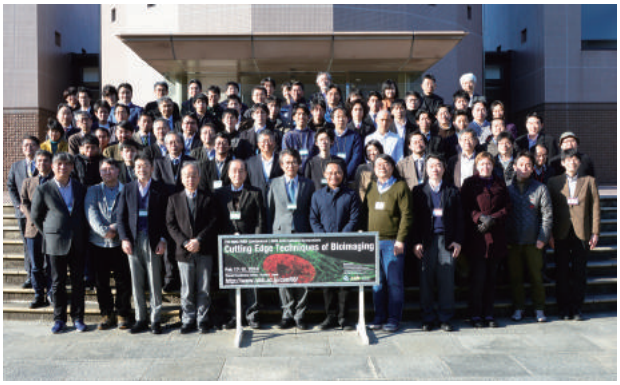
Additionally, much passionate discussion was also exchanged prior to the lecture's Q and A and poster sessions.

A total of 102 participants were able to obtain information on results and analytical techniques concerning the use of advanced bioimaging equipment. They were also able to think more deeply about the usage of advanced imaging techniques in their research. Furthermore, it was also a good opportunity to rethink cooperation in the field of bioimaging with other institutes and researchers.

Due to problems such as budgetary constraints, it is becoming progressively more difficult to introduce advanced equipment and operate it under suitable conditions. Network support activities such as ABiS, which was founded three years ago and has provided advanced bioimaging technologies, are necessary to support bioimaging research in Japan.

In closing, we would like to express our gratitude to the members of NIBB and NIPS for their efforts in hosting this conference.

Shoji Mano (NIBB /ABiS Administration Office)



Speakers

Chew, Teng-Leong (HHMI Janelia, USA), McDole, Kate (HHMI Janelia, USA)
Higashiyama, Tetsuya (Nagoya University, Japan), Okada, Yasushi (The University of Tokyo / RIKEN, Japan), Tamada, Yosuke (NIBB, Japan), Wake, Hiroaki (Kobe University, Japan), Watanabe, Naoki (Kyoto University, Japan)

The 67th NIBB Conference / The 6th Quest for Orthologs Meeting "Quest for Orthologs"

Organizers: Ikuo Uchiyama (NIBB), Christophe Dessimoz (UNIL), Toni Gabaldón Estevan (CRG), Erik Sonnhammer (SU), Paul D. Thomas (USC), Wataru Iwasaki (UTokyo), Shigehiro Kuraku (RIKEN BDR), Shuji Shigenobu (NIBB)

July 31 (Wed)–August 2 (Fri), 2019

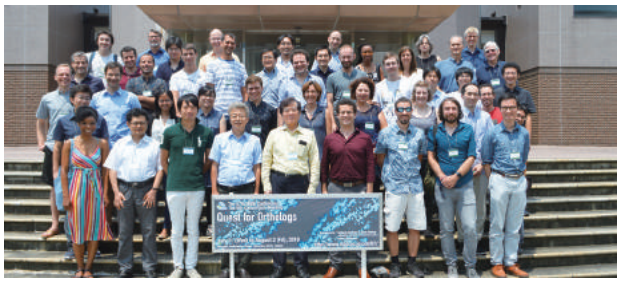
The 67th NIBB conference "Quest for Orthologs" was held with a focus on orthology analysis as a fundamental technology in the field of comparative genomics; something that is becoming more important as genomic technology is applied to a broader range of organisms. This meeting originated from the international consortium of the same name "Quest for Orthologs," which was established in 2009 by developers of orthology inference methods. Since then, the consortium has held a meeting biennially to discuss common subjects in the development of orthology inference methods such as benchmark tests for evaluating methods, a reference sequence set commonly used as input, and a common data format to represent orthology relationships, in addition to presenting the latest results from this field. This meeting, which marked the tenth anniversary of the abovementioned events being held, was hosted in Japan. This was the first time that it had been held outside of Europe and United States, and about half of the participants were from local institutions. As a part of the NIBB conference series, application of orthology inference methods to gene function predictions and/or evolutionary studies with application to biological studies using various organisms were also featured during this meeting.

The conference consisted of four sessions: the development of orthology inference methods and databases, application to function prediction, their application to evolutionary analysis, and new challenges in orthology inference issues. There were 22 invited speakers including 4 keynote lectures, as well as 8 oral presentations and 14 poster presentations by young researchers. During the round table discussion session held at the end of the second day, lively discussions were conducted between developers and users of orthology inference methods.

While this meeting was focused on the specific field of orthology inference methods, it covered a broad range of relevant studies from the basics to specific applications, and provided a good opportunity to review the current and future status of this field as a basis of various genomic studies. A large portion of the event's budget was used to financially support the travel expenses of students and young researchers, who themselves were active contributors to the discussions held during the conference.

We would like to thank all the presenters and participants for helping to stage this conference, the Japanese Society of Bioinformatics and Daiko Foundation for financial support, and the International Cooperation Group of the Research Enhancement Office for organizing the conference.

Ikuo Uchiyama (Laboratory of Genome Informatics)



Speakers

Altenhoff, Adrian (ETH Zurich / SIB Swiss Institute of Bioinformatics, Switzerland), Capella-Gutierrez, Salvador (Barcelona Supercomputing Center, Spain), Dessimoz, Christophe (University of Lausanne, Switzerland), Durand, Dannie (Carnegie Mellon University, USA), Ebersberger, Ingo (Goethe University Frankfurt, Germany), Hansen, Madison (American Museum of Natural History, USA), Jones, Tamsin (EMBL-EBI, UK), Kelly, Steven (University of Oxford, UK), Kriventseva, Evgenia (University of Geneva, Switzerland), Lecompte, Odile (ICUBE/University of Strasbourg, France), Linard, Benjamin (LIRMM, France), Mendes, de Farias Tarcisio (University of Lausanne, Switzerland), Moi, David (UNIL, Switzerland), Nevers, Yannis (ICube laboratory UMR7357, France), Ouangraoua, Aida (University of Sherbrooke, Canada), Patricio, Mateus (EMBL-EBI, UK), Pearson, William (University of Virginia, USA), Sonnhammer, Erik (Stockholm University, Sweden), Szklarczyk, Damian (SIB Swiss Bioinformatics Institute / University of Zurich, Switzerland), Thomas, Paul D. (University of Southern California, USA), Toni, Gabaldón (Centre for Genomic Regulation (CRG), Spain), Warwick, Vesztrocy Alex (University College London, UK), Chiba, Hirokazu (Research Organization of Information and Systems, Japan), Hara, Yuichiro (Nagoya University, Japan), Horiike, Tokumasa (Shizuoka University, Japan), Iwasaki, Wataru (The University of Tokyo, Japan), Kanehisa, Minoru (Kyoto University, Japan), Kuraku, Shigehiro (RIKEN BDR, Japan), Shigenobu, Shuji (National Institute for Basic Biology, Japan), Uchiyama, Ikuo (National Institute for Basic Biology, Japan)

The 2nd NIBB–Princeton Symposium "Imaging and Quantitative Biology"

Organizers: Michael S. Levine (Princeton Univ.), Danelle Devenport (Princeton Univ.), Kazuhiro Aoki (NIBB), Naoto Ueno (NIBB)

October 28 (Mon)–30 (Wed), 2019

The second NIBB-Princeton Joint Symposium, entitled 'Imaging and Quantitative Biology', was held on October 28-30th, 2019 at the Okazaki Conference Center. The National Institutes for Natural Sciences (NINS) and Princeton University have been conducting academic exchange and collaborative research in the life sciences based on an academic cooperation agreement that had been signed by these two parties in 2010.

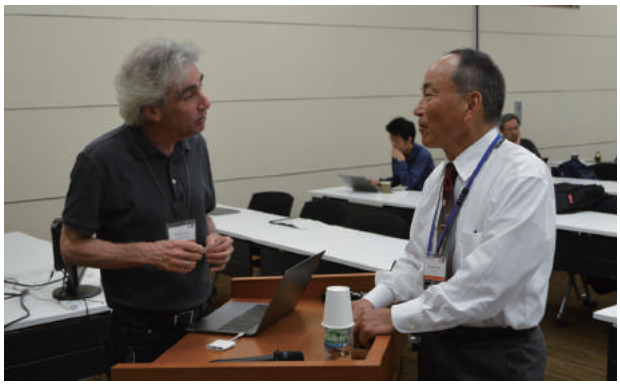
This NIBB symposium was jointly organized not only with Princeton University, but also with the Division of Quantitative and Imaging Biology (QIB) at the International Research Collaboration Center (IRCC), NINS, the National Institute for Physiological Science (NIPS), the Institute for Molecular Science (IMS), and the Exploratory Research Center on Life and Living Systems (ExCELLS), and was supported by the Advanced Bioimaging Platform (ABiS).

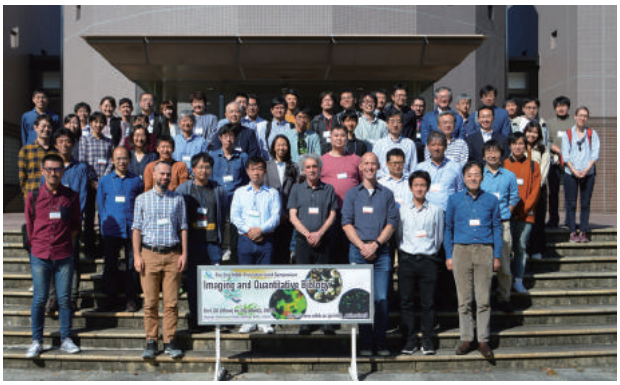
Although the scientific sessions covered a rather broad spectrum of biological phenomena such as molecular dynamics, cell biology, embryogenesis, symbiosis between organisms and viral infection, and information processing within the brain, the key concept 'Imaging and Quantitative Biology' was underlying all the presentations. The latest methodologies such as state of the art imaging technologies, optogenetics, single cell sequencing, mass spectrometry, liquid-liquid phase separation and machine learning presented in the symposium were indeed a showcase of the recent technical advances in life sciences.

Eighty-five participants, who were researchers from the various institutes and research centers of NINS, as well as those from other universities enjoyed lively discussions during poster presentation sessions over the three days of this symposium. In particular, four professors from Princeton University gave insightful comments to the presenters and led the discussions.

In closing, we would like to note that the success of the symposium is something that proves the significant progress of the collaboration between NINS and Princeton University up until now. We would also like to express our gratitude to all participants for taking part in a variety of active discussions, and the IRCC-QIB, the Office of International Cooperation and the individuals from the organizer's laboratories for coordinating and cooperating with this symposium.

Kazuhiro Aoki, Naoto Ueno (On behalf of the organizers)





Speakers

Cristea, Ileana (Princeton Univ., USA), Levine, Michael S. (Princeton Univ., USA), Toettcher, Jared E. (Princeton Univ., USA), Yang, Haw (Princeton Univ., USA)
Aoki, Kazuhiro (NIBB/ExCELLS/IRCC-QIB, Japan), Chikazoe, Junichi (NIPS, Japan), Fujimori, Toshihiko (NIBB, Japan), Fukaya, Takashi (Univ. Tokyo, Japan), Go, Yasuhiro (NIPS/ExCELLS, Japan), Iino, Ryota (IMS/IRCC-QIB, Japan), Kinoshita, Noriyuki (NIBB, Japan), Kitadate, Yu (NIBB, Japan), Morita, Miyo T. (NIBB, Japan), Nemoto, Tomomi (ExCELLS/ NIPS, Japan), Ohsawa, Shizue (Nagoya Univ., Japan), Shigenobu, Shuji (NIBB, Japan), Shiina, Nobuyuki (NIBB/ExCELLS, Japan), Soyano, Takashi (NIBB, Japan), Takada, Shinji (NIBB/ExCELLS, Japan), Takahashi, Shunichi (NIBB, Japan), Uchihashi, Takayuki (ExCELLS/Nagoya Univ., Japan),

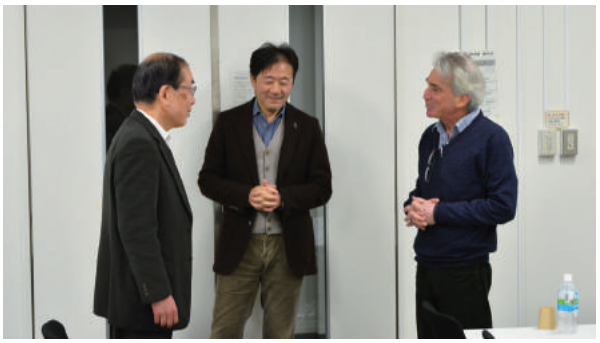
Collaboration Programs with Overseas Institutions

NIBB-Princeton University Collaboration

Professor Michael Levine's visit to NIBB

Professor Michael Levine, the Director of the Lewis-Sigler Institute for Integrative Genomics and professor from the Department of Molecular Biology at Princeton University, visited NIBB from January 15th to 16th 2019 to deliver a seminar and to meet with NIBB researchers. Prof. Levine's visit was supported by NINS's International Research Collaboration Center (IRCC) program to promote international academic exchange between NINS and Princeton University.

Prof. Levine gave a special seminar entitled "Single Cell Resolution of Animal Development" to NIBB researchers. In addition, Prof. Levine had discussions with several NIBB researchers and toured NIBB facilities to observe next-generation sequencers and a light-sheet microscope that has been newly developed at NIBB.



Mr. Dean Edelman's visit to NIBB

Mr. Dean Edelman, the manager of the Office of Corporate Engagement and Foundation Relations at Princeton University, visited NIBB on April 1st 2019. Mr. Edelman's visit was supported by the international academic exchange program between NINS and Princeton University.

Mr. Edelman visited three laboratories affiliated with the NINS's Division of Quantitative and Imaging Biology, International Research Collaboration Center (IRCC-QIB) which are the laboratories administered by Prof. Ryota Iino (IMS), Prof. Kazuhiro Aoki (Division of Quantitative Biology, NIBB) and Prof. Naoto Ueno (Division of Morphogenesis, NIBB). After brief introductions of their respective fields of research, Mr. Edelman discussed future collaborative/cooperative projects between NINS and Princeton University in the field of life science field.

NIBB-EMBL Collaboration

EMBL and the National Institutes of Natural Sciences renew their research exchange agreement.

Since 2005, NIBB has taken a leading role in the academic exchange and collaborative research programs forged between EMBL and the National Institutes of Natural Sciences (NINS), under the auspices of the academic exchange agreement. It has also promoted personal and technological exchanges through symposia, exchanges between researchers and graduate students, as well as the introduction of experimental equipment.

NIBB Director General, Dr. Kiyokazu Agata, and Vice-Director General, Dr. Naoto Ueno, visited EMBL to meet the Director General of EMBL, Dr. Edith Heard from July 1st through 2nd, 2019. The purpose of their visit was to bring a letter renewing the academic exchange agreement between the two institutes signed by Akio Komori, the President of the National Institutes of Natural Sciences. Upon this agreement being signed by Dr. Heard, they exchanged views on international cooperation, collaborative research and the exchange of individuals between the two institutes.

Dr. Ewan Birney's visit to NIBB

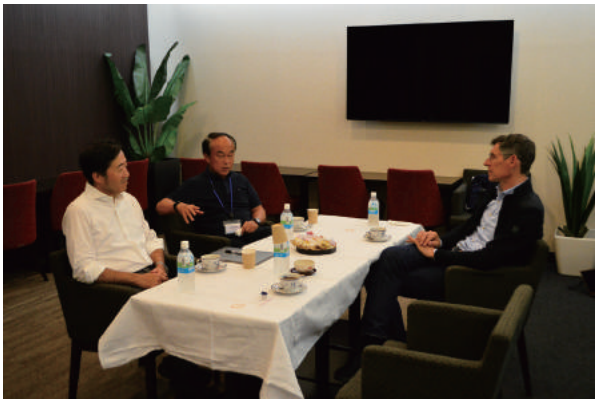
Dr. Ewan Birney, the Director of EMBL-EBI and the senior scientist in charge of research at that institute, visited NIBB on August 21st 2019 to deliver a seminar as well as to meet with NIBB researchers. Dr. Birney gave a special seminar titled "Outbred Genetics in Medaka Fish and Humans - Bringing Models and Medicine Together" to NIBB researchers, and had discussions with the NIBB Director General, Dr. Kiyokazu Agata, to promote an international collaboration between NIBB and EMBL-EBI.

NIBB-COS Collaboration

Prof. Jan Lohmann's visit to NIBB

Starting from 2019FY, NIBB will be entering into an international research collaboration program with the Centre for Organismal Studies (COS) Heidelberg aimed at understanding the adaptation strategies of organisms to environments. Prior to its commencement, the Director of COS, Prof. Jan Lohmann visited NIBB from May 21st through 22nd, 2019 to discuss upcoming international collaborative research projects and international cooperation between both institutes with NIBB Director General, Dr. Kiyokazu Agata, and Vice-Director General, Dr. Naoto Ueno.

Prof. Lohmann introduced COS and presented his research entitled 'Signal Integration in Plant Stem Cells' to NIBB researchers and graduate students. In addition, he toured the NIBB facilities while exchanging opinions with fellow researchers. This in turn contributed to a closer relationship between our institutions.



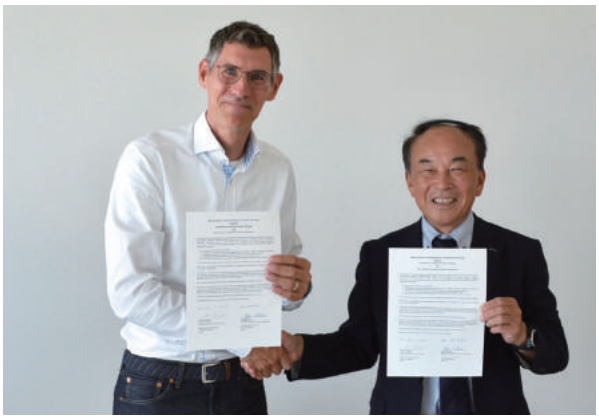
Dr. Alexis Meizel's visit to NIBB

Dr. Alexis Meizel from COS Heidelberg visited NIBB on May 30th, 2019 during his research posting at Nagoya University. He had a discussion on collaborative research projects using the IR-LEGO system with Associated Prof. Yasuhiro Kamei and Dr. Joe Sakamoto (NIBB Research Fellow) at the Spectrography and Bioimaging Facility.

NIBB signs academic exchange agreement with COS Heidelberg, Germany

NIBB has signed an academic exchange agreement with COS Heidelberg, Germany. NIBB and COS Heidelberg have conducted collaborative research in several research fields using small fish, cnidarians, plants and microscopies. In addition to this, researchers and graduate students from both institutes have previously visited the other for the purposes of this collaboration.

NIBB Director General, Dr. Kiyokazu Agata, and Vice-Director General, Dr. Naoto Ueno, visited COS Heidelberg from July 1st through 2nd, 2019 to sign an agreement facilitating academic exchange between the institutes. They also used this opportunity to present their research projects during seminars for COS researchers and students, as well as to exchange ideas concerning future collaborative/cooperative programs with COS PIs including the COS Managing Director, Prof. Dr. Jan Lohmann.



Dr. Annika Guse's visit to NIBB

NIBB will undertake an international research collaborative program with COS Heidelberg aimed at understanding the adaptation strategies of organisms to environments. In this program, researchers who belong to NIBB and COS Heidelberg will start a collaborative research program focusing on the mechanisms and evolution of light sensing in cnidarians. Dr. Annika Guse, who is currently analyzing the mechanisms and evolution of light sensing in cnidarians, visited NIBB from September 8th through 9th, 2019 regarding this.

On this occasion, she gave a presentation entitled Molecular Mechanisms of Coral-Algal Endosymbiosis, and was able to strengthen her relationship with multiple NIBB researchers via academic discussion.

Collaborative Activities with Joint Usage / Research Center Domestic Institutions

NIBB Signs Agreement of Cooperation with Institute of Low Temperature Science, Hokkaido University (ILTS)

NIBB has signed an agreement with the Institute of Low Temperature Science, Hokkaido University (ILTS) aimed at promoting collaboration and cooperation between these two institutions. A signing ceremony was held at NIBB on December 9, 2019.

NIBB researchers and ILTS researchers have been working together on several collaborative projects, NIBB and ILTS have been engaged in cooperative and collaborative research activities within their respective standard operations for some time, and both institute NIBB and ILTS aim to further develop research in the field of low-temperature science and basic biology with the signing of this agreement.

Dr. Kiyokazu Agata, the Director General of NIBB, and Dr. Manabu Fukui, the Director of ILTS, signing the agreement.

Following the signing ceremony, the Director of ILTS, Prof. Manabu Fukui, provided an outline of ILTS. Additionally, Prof. Fukui and Prof. Yoshifumi Yamaguchi of ILTS gave seminars concerning their research project. The signing ceremony and seminars were attended by a large number of NIBB members, and provided an opportunity for friendly exchanges to occur which will hopefully promote further cooperation and collaboration.

NIBB Priority Collaborative Research Projects

- Molecular and physiological mechanisms for understanding mammalian hibernation and their comparative analysis among species
  Prof. Yoshifumi Yamaguchi (ILTS)
  Prof. Shuji ShigenobuToshihiko Fujimori (NIBB)
  Prof. Shuji Shigenobu (NIBB)

The 13th NIBB Bioimaging Forum "Behavioral and Recognition Research upon the Platform of Vision and Color-Recognition Studies and its Interdisciplinary Merging with Bioimaging"

Organizing Committee: Yasuhiro Kamei, Shoji Fukamachi, and Hideaki Takeuchi

February 12 (Tue)–13 (Wed), 2019

The 13th NIBB Bioimaging Forum was held in conjunction with the achievement briefing meeting conducted by the priority collaborative research project led by Prof. Fukamachi entitled "Toward Construction of a Research Platform of Vision and Color-Recognition Studies -Upon the Model of Medaka Whose Behaviors are Dominated by Vision- ". This collaborative research project was aimed at establishing methodologies for analyzing issues ranging from individual behaviors to molecular mechanisms of vision, which also use imaging technologies such as virtual reality and aerial imaging. As bioimaging aims to improve observation technologies, this joint meeting was viewed as a good opportunity for exchanging study results and other information.

In particular, rhodopsin (and other opsins) is a key substance combining the two participant groups. This is due to it being directly related to vision. It also can be used as a tool in optogenetics, itself a cutting-edge bioimaging methods. Additionally, Medaka have been used as a model organism for more than a hundred years in Japan, and have become popular as pets due to the many color variations they produce through cross-breeding.

We made this symposium open to public for the purposes of disseminating of knowledge on the research uses of Medaka and their history as a model organism, something which has likely benefitted historically. It was unfortunate that two of the invited speakers withdrew due to cancelling caused by an influenza epidemic, but their absence was covered by two young participants in a similar research field. This shows a large pool of researchers active in this field.

I would like to thank all the speakers and the project members for their contribution.

(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 2019, we held three sets of training courses on RNA-seq analysis. Each set 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 sequencing data. 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, Mr. Hiroki Sugiura (NIBB Core Research Facilities)

February 21 (Thu)–22 (Fri), 2019

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

- 30 participants (including 2 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 14 (Thu)–15 (Fri), 2019

(Practical Course) RNA-seq Analysis Pipeline

- 28 participants (including 1 from NIBB)
- 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

May 16 (Thu)–17 (Fri), 2019

(Preparatory Course) Basics of UNIX, R, and NGS
28 participants (including 3 from NIBB)

May 30 (Thu)–31 (Fri), 2019

(Practical Course) RNA-seq Analysis Pipeline
30 participants (including 4 from NIBB)

May 16 (Thu)–17 (Fri), 2019

(Preparatory Course) Basics of UNIX, R, and NGS
26 participants (including 6 from NIBB)
* Practical Course was postponed due to the COVID-19.

The 7th Bio-imaging Data Analysis Training Course

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

December 10 (Tue)–12 (Thu), 2019

The 7th Bio-imaging Data Analysis 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. 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”. Forty three people applied for the course, which had an announced capacity of 16 participants. Considering the high demand for courses on these subjects, we accepted 18 participants.

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)

The Center for the Development of New Model Organisms Training Course "Technical Workshop on Microinjection Techniques Using New Model Organisms"

Instructors: Ken-ichi Suzuki, Shuji Shigenobu, Teruyuki Niimi, Kiyoshi Naruse
Lecurers: Ken-ichi Suzuki, Tatsuma Mohri, Masato Ohtsuka
Organizers: Ken-ichi Suzuki, Shuji Shigenobu, Naoto Ueno

The development of new model organisms has enabled the outstanding recent progress of genome sequencing and editing technologies. In keeping with this, the stable rearing and reproduction of animals, the preparation of genetic information, and especially the establishment of experimental protocols for functional analyses is necessary for the establishment of new model organisms. Microinjection is a fundamental technique found among these protocols, which itself is used in many functional operations, *e.g.* genome editing and RNAi. However, the development of microinjection techniques, presents an obstacle to developing new organisms, due to these type of techniques requiring intensive optimization for each new organism. With this in mind, The Center for the Development of New Model Organisms of NIBB organized a technical workshop during 2019 concerning microinjection techniques in order to promote and share said techniques among the scientific community.

We welcomed fourteen participants from various backgrounds, including academic researchers, students, and researchers from private companies. Participants were divided into three groups, each of which worked for two days with either amphibians (Iberian ribbed newt), insects (aphids and silverfish), or fish (medaka). Participants learned and practiced the basics of injection techniques as they were optimized for each organism. They ultimately joined together for a debriefing session where their individual experiences were shared. We also had a lecture session regarding functional analyses techniques by the application of microinjection. Four lectures, that were mostly run by invited extramural researchers, outlined the history of microinjection and genome editing. Researchers from a private company developing and manufacturing microinjection instruments also joined in with these sessions, thereby facilitating an informative exchange between users and developers.

This course successfully provided a valuable opportunity for participants to share microinjection techniques. One of the participants said those present could “acquire skills not provided in published textbooks or papers. As such, I felt the course was very informative". Another attendee stated that they "could exchange information with lecturers and other participants, and felt the conversations I was able to have were valuable". Although microinjection is a classic technique, it no doubt grows even more important as it is required in the application of cutting-edge technologies such as genome editing. We at the Center for the Development of New Model Organisms, NIBB, continue to focus on the improvement and promotion of this both old yet new technique, while keeping an eye on the collective needs of researchers.

(Shuji Shigenobu)

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, increasingly complex operations, increased equipment and running costs, as well as the growing need for image data analysis, individual research institutes and universities are increasingly encountering difficulties related to the introduction, maintenance, and operation of imaging equipment.

ABiS was launched as one of the designated "Platforms for Advanced Technologies and Research Resources" during fiscal year (FY) 2016 under the new framework of the Grant-in-Aid for Scientific Research on Innovative Areas (Leader: Prof. Masanobu KANO, NIPS/The University of Tokyo). This program aims to contribute to the further development of academic research in Japan through the provision of cutting-edge equipment and methodologies to individual KAKENHI (Grants-in-Aid for Scientific Research) research projects by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) under the Grant-in-Aid for Scientific Research on Innovative Areas (FY2016-FY2021).

Cooperating with domestic partner organizations that own and operate multiple types of advanced specialized imaging equipment, ABiS provides cutting-edge instruments for light microscopy, electron microscopy, magnetic resonance imaging, and other methods through its network. It aims to provide comprehensive support for advanced imaging in the field of life science. NIBB, together with NIPS, contributes as a core institute in the ABiS network. Among the various support activities that ABiS performs, NIBB is tasked with the following:

- 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, Assist. Prof. Kagayaki KATO and Assist. Prof. Yusaku OHTA.
- Training
  - Training for image analysis administered by Assist. Prof. Hiroshi KOYAMA and Prof. Naoto UENO

To organize and coordinate ABiS activities, two secretariat offices were established at NIBB (Assoc. Prof. Shoji MANO) and NIPS, respectively, under the control of the general support group (Individuals in charge at NIBB; Director-general Prof. Kiyokazu AGATA, Prof. Naoto UENO, Prof. Shinji TAKADA, Assoc. Prof. Shoji MANO). General support provided includes budget planning and management of ABiS activities. In particular, we promote ABiS

activities via its associated website and other media, so that KAKENHI researchers make full use of the ABiS platform to accelerate their research projects. In addition, we organize technology training sessions, workshops, and symposia to disseminate advanced imaging technologies and share information about them. We also coordinate with the other three platforms (Platform of Supporting Cohort Study and Biospecimen Analysis, Platform of Advanced Animal Model Support, and Platform for Advanced Genome Science) to provide both multidisciplinary and international support.

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 areas such as India, Australia, Singapore, the Republic of South Africa, Canada, Mexico, USA, and some Latin American countries. In 2018, ABiS joined the GBI project representing the Japanese bioimaging community. It is hoped that through GBI, ABiS will be able to raise the quality of present support to that of the currently accepted international benchmarks, and that further observations, data analyses and research methods will be able to be better shared globally. Furthermore, it is also hoped that this collaboration will lead to set up a better environment for bioimaging research in Japan. This will be achieved not only by providing international training courses for young researchers and the staff of imaging facilities, but also through discussions about the implementation of career paths; a common problem in this field.

GBI also aims to establish an image data sharing infrastructure system, promote academic/industrial cooperation and formulate a global strategy for bioimaging research. It has been decided that the GBI event "The Fifth Exchange of Experience (EoE)" hosted by ABiS, will be held in Okazaki, Japan during the autumn of 2020.

Image Competition Held During The 57th Annual Meeting of The Biophysical Society of Japan (BSJ)

September 24 (Tue)–26 (Thu) 2019

ABiS held the 'ABiS Image Competition' as a special joint event in collaboration with BSJ during the 57th annual meeting of BSJ in Miyazaki (Chair: Dr. Takeharu NAGAI) to encourage researchers, especially students, graduate students, and young researchers, to present their work to the research community. Winners were selected from the categories of 'Humor', 'Intellectual' and 'Beauty' on the basis of votes collated from attending participants, and were publicly commended by ABiS and the 57th Annual meeting of BSJ (Figure 1). All photographs and videos are available to the public on the annual meeting's website (https://www2.aeplan.co.jp/bsj2019/abisicontest/57_abisimagecontest_list).

Figure 1. Award giving ceremony. Prof. UENO (Center) awarding prizes to winners of the image competition. Osaka University's Prof. Takeharu NAGAI (Left) chaired the meeting.

Training Course for AI-based Bio-image Analysis

August 29 (Thu)–30 (Fri) 2019

In addition to the support given by cutting-edge imaging research technologies, ABiS holds various training courses for image acquisition, processing and analysis to further disseminate imaging techniques every year. ABiS held 10 training courses on light microscopy, immunoelectron microscopy, MRI and imaging analysis in 2019. Among them, AI-based bio-image analysis was held at Kumamoto University, which was organized by Assoc. Prof. Takumi HIGAKI (Kumamoto Univ.), Assist. Prof. Kagayaki KATO (NIBB), Assist. Prof. Yusaku OHTA (NIBB) and Prof. Naoto UENO (NIBB). Prof. Seiichi UCHIDA (Kyushu Univ.) and Prof. Akira FUNAHASHI (Keio Univ.), who are recognized for their expertise in software and algorithm creation in the field of imaging analysis, and AI-based systems biology, respectively, were invited lecturers (Figure 2).

Figure 2. Training course session at Kumamoto University. Fifteen participants were selected via examination of their respective applications, and received lectures on AI-based machine learning for bio-image analysis.

The NIBB Internship Program

The NIBB Internship program, which started in 2009, is a hands-on learning course for overseas students designed to give high-quality experience in real world research and a focused education in biology. At the same time, this program aims to internationalize graduate students from The Graduate University for Advanced Studies SOKENDAI, giving them the opportunity to get to know students and interns with differing 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 2019 there were 29 applicants, out of which 11 interns were selected. These interns were from universities located in six countries (Vietnam, Thailand, Germany, Hungary, USA, and Japan) and spent periods ranging from two to twelve weeks experiencing life as a member of a research team. Moreover, two interns from the UK and Germany, who were funded by travel grants they had applied for, were also posted to NIBB.

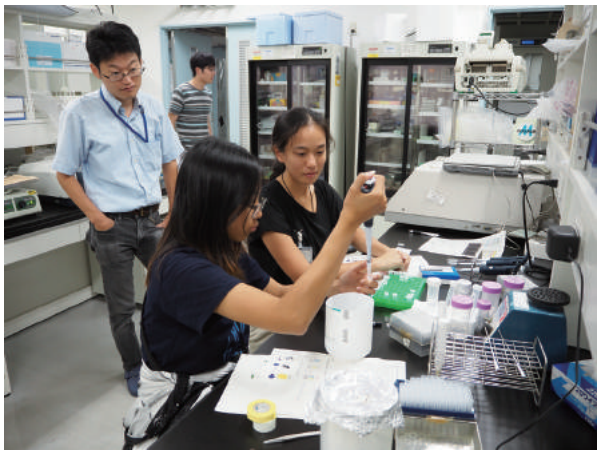
Report from a participant
Neen Phan-udom
Mahidol University, Thailand

My name is Neen Phan-udom. I am a third-year undergraduate student from Mahidol University Thailand, majoring in Bioresources and Environmental Biology (International Program). I had the opportunity to do a 2-week internship at Professor Ueda's lab- the Division of Cellular Dynamics- which works on plant membrane trafficking proteins in *Arabidopsis thaliana* and *Marchantia polymorpha*.

I was under the supervision of Asst. Prof. Ebine during my internship. My work mainly consisted of performing transient assays for *Arabidopsis thaliana* leaves and visualizing the localization of the targeted proteins via confocal microscopy. I learned to find and take pictures of the cells expressing GFP/RFP-tagged proteins to later measure their activities and possible interactions. I also got to attend a 2-day summer practical course on oil bodies in liverwort which included mini fieldwork to collect liverwort samples and visualize their oil bodies under the microscope.

In addition to gaining more knowledge and laboratory skills, this internship was one of the most wonderful experiences I've ever had. I received a very warm welcome from all the lab members as well as other staff at NIBB. I was pleasantly surprised when the lab organized a welcoming party for me and my lab partner on our first day (which has gotten me really into soumen). Everyone was so nice and friendly which made me feel very welcomed and look forward to my tasks every day. I was on the verge of crying on my last day here when I had to say goodbye. I also had many wonderful moments learning about Japanese culture both inside and outside of the lab, not to mention having the privilege to live in Okazaki city and do some traveling on the weekends. Japan really is a beautiful country.

I will never forget my two weeks here. If anything, I wish I could have stayed longer and learned more about membrane trafficking in plants. The internship has shown me the beauty of basic research of exploring the unknowns and left me wanting for more. Really, I can't thank everyone enough for this amazing experience. I am forever grateful for this internship opportunity.



The NIBB Openhouse 2019

October 5 (Sat), 2019

The triennial NIBB Open House was held on 5 October 2019, and was attended by 2,980 people.

This event saw us opening our laboratories and facilities in the Myodaiji area to the public in order to introduce cutting-edge biological research.

Talks Live

The event combined talks and demonstrations run by NIBB researchers. Four researchers, including the Director General Kiyokazu Agata, gave talks about why living things are so fascinating, which were followed by a demonstration and Q&A session with the audience.

Booth Exhibits

There were 31 laboratories or departments in two separate venues:, the NIBB main building and the conference hall.

Exhibition of the Nobel Prize Monument and Nobel Medal (official replica) of dedicated to Professor Emeritus Yoshinori Ohsumi

Science Workshop and Research Poster Exhibit by Okazaki High School SSH Club

Stamp Rrally

Unique badges were given to visitors who had collected a designated number of stamps at various locations within the Open House event.

Outreach Activities

NIBB widely disseminates our research and results to the general public, and we conduct activities to convey the excitement of studying biology to students at elementary, junior high, and high schools to nurture the next generation of scientists.

Visits to schools

July 2 (Tue), 2019

Special class at Kouzan Junior High School

■ Assist. Prof. Keiko Nonomura

July 10 (Wed), 2019

Special class at Nukata Junior High School

■ S. A. Assist. Prof. Tomoko Kurata

July 11 (Thu), 2019

Special class at Yahagi Junior High School

■ Assist. Prof. Ryutaro Tokutsu

July 18 (Thu), 2019

Special class at Iwazu Junior High School

■ Assist. Prof. Yusuke Mii

October 24 (Thu), 2019

Special class at Ryunan Junior High School

■ Prof. Teruyuki Niimi

November 14 (Thu), 2019

Special class at Mikawa Junior High School

■ S. A. Assoc. Prof. Kenji Takizawa

December 3 (Tue), 2019

Special class at Aoi Junior High School

■ Prof. Teruyuki Niimi

January 14 (Tue), 2020

Special class at Okazaki High School

■ Director General Kiyokazu Agata

December 20 (Fri), 2019

Special class at Aichi Sangyo University Mikawa High School

■ Assoc. Prof. Shigenori Nonaka

June 19 (Wed), 2019

Feedback session regarding research presentations by high school students

■ S. A. Assist. Prof. Kiyoshi Tatematsu

December 27 (Fri), 2019

Feedback session regarding research presentations by high school students

■ S. A. Assist. Prof. Kiyoshi Tatematsu

Seminars for school teachers

October 29 (Tue), 2019

■ Assist. Prof. Atsushi Hoshino

Work experience for junior high school students at NIBB

■ Honan Junior High School students
Ryujin Junior High School students

Programs broadcast live online

August 23 (Fri)–31 (Sat), 2019

"Observing the Iberian ribbed newt developmental process over a period of 200 hours"

This was a 200 hour live broadcast displaying the developmental process of the Iberian ribbed newt. Researcher commentaries were provided via the live internet broadcast platform, NicoNico

■ S. A. Assoc. Prof. Ken-ichi T. Suzuki
Dr. Miyuki Suzuki
Director General Kiyokazu Agata
Assist. Prof. Yuki Moriyama (Chuo Univ)
Prof. Toshinori Hayashi (Hiroshima Univ.)
S. A. Assist. Prof. Tomoko Kurata

■ Number of accesses: 452174

Lectures for the public

June 2 (Sun), 2019

Lecture at the Natural History Museum/Sea of Life

■ Prof. Teruyuki Niimi

Workshop at Natural History Museum/Sea of Life

■ Dr. Tatsuro Konagaya
S. A. Assist. Prof. Tomoko Kurata

April 16 (Tue), 2019

Open lecture to commemorate the appointment of the Director General of NIBB

■ Director General Kiyokazu Agata

October 20 (Sun), 2019

Inter-University Research Institute Corporation Symposium 2019

■ Prof. Shuji Shigenobu

July 7 (Sun), 2019

National Institute of Natural Sciences
The 8th Young Researcher Award Commemorative Lecture

■ Assist. Prof. Yu Kitadate

February 8 (Fri), 2020

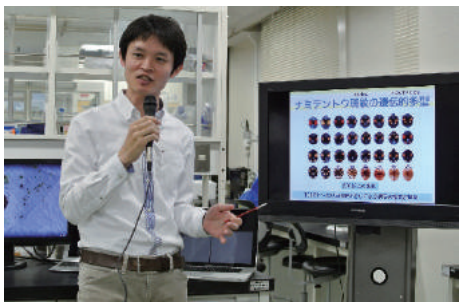
Presentation by OKASHIN (Okazaki Credit Union) scholarship students

- Dr. Mariko Kishimoto
- Special lecture at OKASHIN
  Director General Kiyokazu Agata

March 11 (Wed), 2020

Online lecture

- Assist. Prof. Toshiya Ando



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

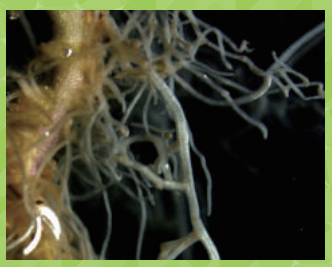
Take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes from the airport).

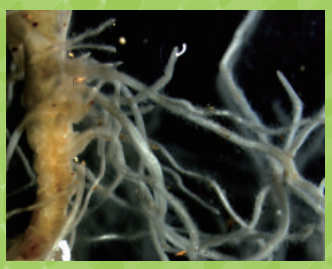
From Higashi Okazaki Station to Each Area

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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