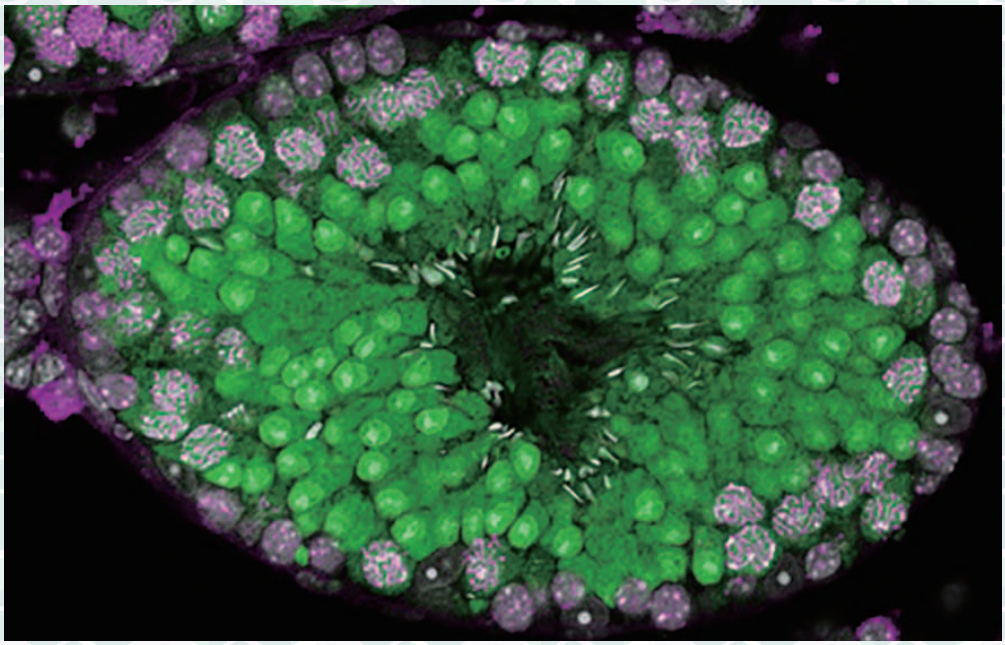
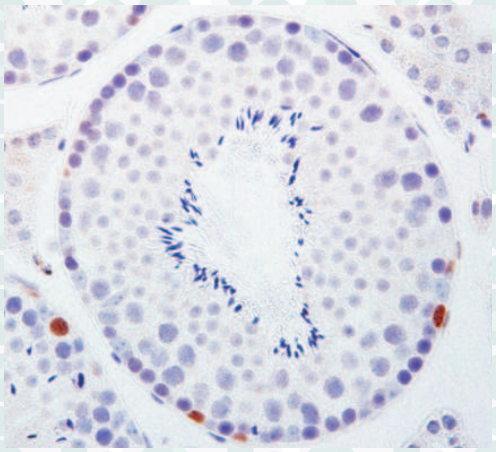
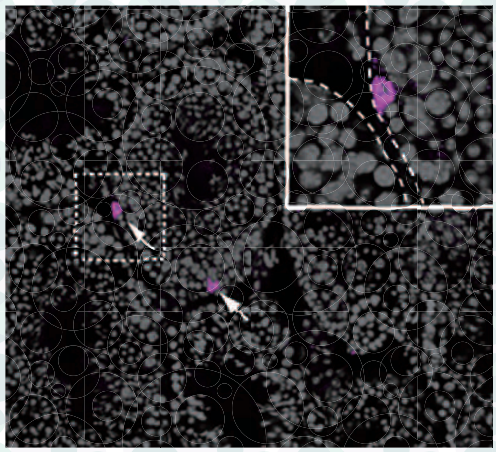






National Institute for Basic Biology

2021 ANNUAL REPORT

April 2021–March 2022

CONTENTS

The cover items are related to and reproduced with modifications from the following papers from the Division of Germ Cell Biology: “Transient suppression of transplanted spermatogonial stem cell differentiation restores fertility in mice” (Nakamura *et al*., Cell Stem Cell 2021, https://doi.org/10.1016/j.stem.2021.03.016) and “A multistate stem cell dynamics maintains homeostasis in mouse spermatogenesis” (Nakagawa *et al*., Cell Reports 2021, https://doi.org/10.1016/j.celrep.2021.109875). See page 36 for details.

It is my great pleasure to present all the National Institute for Basic Biology (NIBB)'s 2021 Annual Report which outlines NIBB's research, educational, and international activities and its effective function as a center for collaborative research in Japan from April 2021 to March 2022.

The NIBB's research activities have largely resumed to normal in 2021, following the significant easing of restrictions caused by the COVID-19 pandemic.

Visits of researchers from overseas and travel abroad by NIBB researchers are gradually returning to previous levels. Online exchange activities are also continuing. The 2nd and 3rd Meetings for NIBB-COS Heidelberg International Collaborations were held online.

In 2021, NIBB signed a new collaboration agreement with the Institute for Molecular and Cellular Regulation, Gunma University. Joint research on intracellular membrane trafficking is being conducted between the two institutes. In addition, We also initiated the seminar series "AI and Biosystems" with the aim of active participation of AI in biological research in collaboration with Chubu University and the National Institute for Physiological Sciences.

The well-equipped core facilities that support research activities are one of the notable features of NIBB. It was truly pleased that Ms. Tomoko Mori, Ms. Yumiko Makino, Mr. Katsushi Yamaguchi, and Mr. Takahiro Bino, technical staff of the Functional Genomics Facility, were awarded the Outstanding Support for Research Award, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science, and Technology, for their contribution to research support. Furthermore, Professor emeritus Yoshitaka Nagahama was awarded The Order of the Sacred Treasure, Gold Rays with Neck Ribbon by the Japanese government. We extend our sincere congratulations on this honorable award for his many years of outstanding achievements.

We are grateful for our status as a leading international research institute in the field of basic biology, we are constantly striving for improvement and welcome your valuable feedback. We would like to express our sincere gratitude for your continued support.

Kiyokazu Agata
Director General of NIBB
December, 2022

ORGANIZATION OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

The National Institute for Basic Biology (NIBB) was founded in 1977 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 biology, developmental biology, neurobiology, evolutionary biology, environmental biology, and theoretical biology.

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

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.

Policy, Decision Making, and Administration

The Director General oversees the operation of NIBB and is 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 collaborative research programs as well as on the scientific activities of NIBB.

The Research Enhancement Strategy Office was founded in 2013 and aims to support researchers to improve NIBB's abilities as a collaborative research institution.

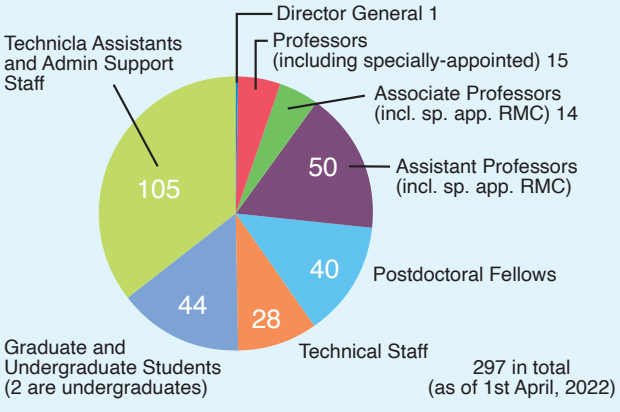
Administration of NIBB is undertaken by the Okazaki Administration Center under the direct auspices of the Administration Office of NINS.

Research and Research Support

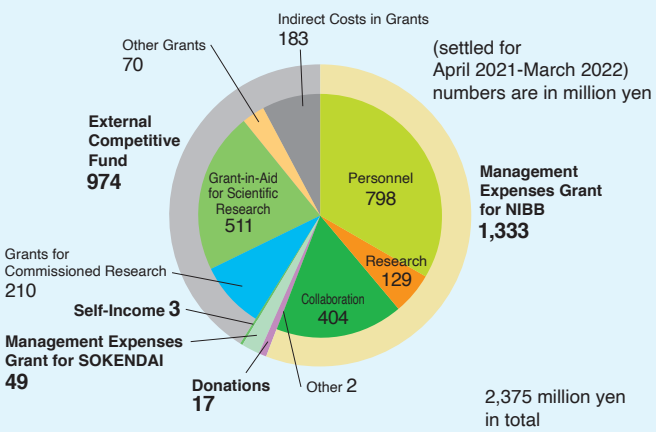
NIBB's research programs are conducted in research units called divisions and laboratories. Each research unit forms an independent project team. The NIBB Core Research Facilities and NIBB BioResource Center were launched in 2010 to support research at NIBB and also to act as an intellectual hub to promote 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. The Center for the Development of New Model Organisms was founded in 2013 to promote the development of new model organisms and research that uses them. The Technical Division manages the activities of the technical staff and helps to promote the activities of each research unit and research support facility to maintain the common resources of NIBB and NINS.

The Astrobiology Center (ABC) was established in 2015 as a NINS research center. Additionally, the Exploratory Research Center on Life and Living Systems (ExCELLS) was founded in 2018. Several NIBB researchers are also affiliated with ABC and ExCELLS.

Members in NIBB



Financial Configuration of NIBB



Organization

National Institute of Natural Sciences (NINS)

President
KOMORI, Akio

- National Astronomical Observatory of Japan (NAOJ)
- National Institute for Fusion Science (NIFS)
- National Institute for Basic Biology (NIBB)
  Director General
  AGATA, Kiyokazu
  Vice-Director General
  HASEBE, Mitsuyasu
- National Institute for Physiological Sciences (NIPS)
- Institute for Molecular Science (IMS)

Three Institutes in Okazaki

- Center for Novel Science Initiatives
  - Frontier Photonic Science
  - Plasmabio Science
- Astrobiology Center
- Exploratory Research Center on Life and Living Systems (ExCELLS)
  - Department of Creative Research
  - Section for Exploration of Life in Extreme Environments

  Research units of NIBB with an asterisk on the right panel also function as research groups of ExCELLS.
- International Research Collaboration Center (IRCC)

Advisory Committee for Programming and Management

Research Enhancement Strategy Office
- Evaluation and Information Group
- Public Relations Group
- Liaison and Coordinaton Group
- Collaborative Research Group
- Young Researcher Support Group
- Gender Equality Promotion Group
- Collaborative Innovation Group

Okazaki Research Facilities
- Center for Radioisotope Facilities
- Center for Animal Resources and Collaborative Study
- Research Center for Computational Science

Research Facilities run jointly by NIBB and NIPS
- Electron Microscopy Room
- Waste Management Room
- Instrument Design Room

Okazaki Administration Center

National Institute for Basic Biology (NIBB)

As of March 31, 2021

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

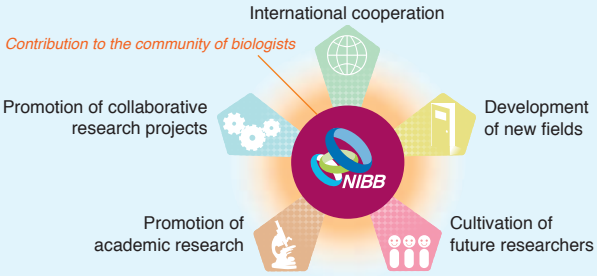
Members of the Advisory Committee for Programming and Management (terms of April 2021 to March 2023)

External committee	KITANO, Jun	Professor, National Institute of Genetics
	KYOZUKA, Junko	Professor, Tohoku University
	KUROIWA, Asato	Professor, Hokkaido University
	SATAKE, Akiko	Professor, Kyushu University
	SIOMI, Mikiko C.	Professor, Tokyo University
	NIWA, Hitoshi	Professor, Kumamoto University
	HANASHIMA, Carina	Associate Professor, Waseda University
	HIGASHIYAMA,Tetsuya	Professor, Tokyo University
	FUKUI, Manabu ##	Professor, Hokkaido University
	YOSHIMURA, Takashi	Professor, Nagoya University
Internal committee	UEDA, Takashi	Professor, National Institute for Basic Biology
	MORITA, Miyo T.	Professor, National Institute for Basic Biology
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, National Institute for Basic Biology
	NIIMI, Teruyuki	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	HIGASHIJIMA, Shin-ichi	Professor, National Institute for Basic Biology
	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	MINAGAWA, Jun #	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

In the pursuit of progress in the field of biology, The National Institute for Basic Biology (NIBB) has set five goals regarding its activities. We consequently contribute to the world-wide community of biologists through our efforts to achieve these goals.



Promotion of academic research

One of our goals is the promotion of academic research, and this is accomplished through our research activities, which we will introduce throughout this brochure.

Promotion of Collaborative Research Projects

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 when visiting NIBB's laboratories to conduct collaborative research. The "Collaborative research projects for new model organism development" and "Collaborative Research Projects for Bioresource Preservation Technology Development" projects are for developing and establishing new model organisms and new research technology. Research and travel expenses are provided for these projects. 'Collaborative Research Projects for Integrative Genomics' and 'Collaborative Research Projects for Integrative Bioimaging' are projects to facilitate a 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	2019	2020	2021
Priority collaborative research projects	1	1	—
Collaborative research projects for new model organism development	—	2	3
Collaborative research projects for model organism and technology development	2	—	—
Individual collaborative research projects	60	46	42
Collaborative research projects for integrative genomics	66	56	58
Collaborative research projects for integrative bioimaging	22	19	24
NIBB workshops	3	2	4
Collaborative experiments using the Large Spectrograph	9	9	11
Support for NIBB training courses	0	0	0
Collaborative research projects for bioresource preservation technology development	14	10	10
Total	177	145	152

National BioResource Project

The National BioResource Project (NBRP) is a national project for the systematic accumulation, storage and supply of nationally recognized bio-resources (*i.e.*, 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 became available. NIBB is also a sub-center for the NBRP's work with Japanese morning glories and Zebrafish (p. 101-102).

Inter-University Bio-Backup Project for Basic Biology (IBBP)

To prevent damage caused by natural disasters to important biological resources, NIBB has managed the Inter-University Bio-Backup Project for Basic Biology (IBBP) from 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources used in cutting-edge research (p. 103-104).

Advanced Bioimaging Support (ABiS)

ABiS provides assistance pertaining to advanced imaging in research supported by Grants-in-Aid for Scientific Research. NIBB and 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 to represent the Japanese bioimaging community. ABiS also hosts training courses (p. 116-117).

International Cooperation

Collaborative Programs with Overseas Institutes

NIBB has formed an agreement with Center for Organismal Studies (COS) Heidelberg to promote joint research projects, collaborative symposia, and student exchange programs. The 2nd and 3rd meeting for NIBB-COS Heidelberg Internatinal Collaborations were held in June, 2021 and March, 2022 (p. 112). In these meeting, several discussions on new collaborative research were put forward.

NIBB plays a leading role in collaborative research programs between Princeton University and the National Institutes of Natural Sciences (NINS) on the life science research field. Collaborative research projects between NIBB and Princeton University are conducted under the support of the International Research Collaboration Center (IRCC) of the NINS (p. 112). NIBB also promotes personal and technological exchange with the European Molecular Biologiy Laboratory (EMBL) based on the academic exchange agreement between the EMBL and the NINS.

NIBB Conference

The NIBB Conferences are international conferences on prominent topics in biology that are organized by NIBB professors. Since the first conference in 1977 (the year of NIBB's founding), NIBB Conferences have provided researchers in basic biology with valuable opportunities for

international exchange. Unfortunately, this year's NIBB conference was cancelled due to the COVID-19 pandemic.

Development of New Fields of Biology

Establishment of new model organism

NIBB is working to establish novel model organisms in order to advance research on biological phenomena that are interesting but have been poorly analyzed.

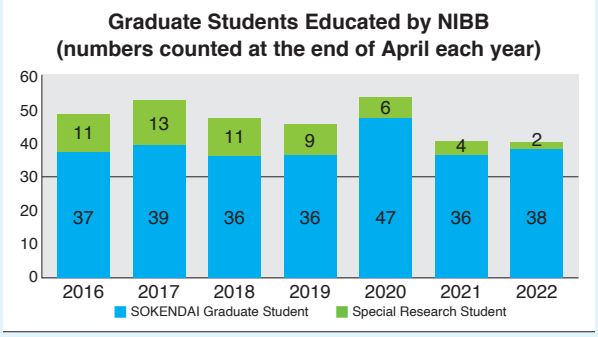
Cultivation of Future Researchers

Ph.D. program

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 doctoral course for university graduates and a three-year doctoral course for graduate students with a master's degree. Additionally, graduate students enrolled in other universities can apply to be special research students eligible to conduct research under the supervision of NIBB professors. In any case, 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 its international collaboration with EMBL, graduate students are encouraged to attend PhD student symposia held at EMBL at least once during their 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 institute (p. 118).



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 (*i.e.* web pages, Facebook, and Twitter). NIBB also cooperates in the education of undergraduate and young students through lectures and workshops (p.119-123).

Personnel changes from April 2021 to March 2022*

Newly assigned to NIBB

Name	Position	Research Unit	Date
SUZUKI, Shinnosuke	Specially Appointed Assistant Professor	Division of Germ Cell Biology	April 1, 2021
OKUMA, Nao	Specially Appointed Assistant Professor	Division of Symbiotic Systems	April 1, 2021
YOKONO, Makio	Specially Appointed Assistant Professor	Division of Environmental Photobiology	July 1, 2021
MORITA, Shinichi	Specially Appointed Assistant Professor	Division of Evolutionary Developmental Biology	October 1, 2021
ARATA, Masaki	Specially Appointed Assistant Professor	Division of Embryology	October 1, 2021
KAWAGUCHI, Takayuki	Assistant Professor	Division of Chromatin Regulation	November 1, 2021

Change of Status in NIBB

Name	New Affiliation	Position	Date
NONOMURA, Keiko	Associate Professor	Division of Embryology	January 1, 2022
YOKONO, Makio	Associate Professor	Division of Environmental Photobiology	February 1, 2022

Newly affiliated with other universities and institutes

Name	New Affiliation	Position	Date
NONOMURA, Keiko	Tokyo Institute of Technology	Associate Professor	January 1, 2022
SHINOZUKA, Takuma	Nara Institute of Science and Technology	Assistant Professor	January 1, 2022
ANDO, Toshiya	Kyoto University	Program-Specific Associate Professor	April 1, 2022
SAITO, Nen	Hiroshima University	Associate Professor	April 1, 2022

Mandatory Retirement

Name	Position	Research Unit	Date
UENO, Naoto	Professor	Division of Morphogenesis	March 31, 2022

Awardees from April 2021 to March 2022

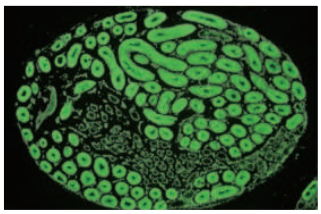
Name	Position	Award
MORI, Tomoko	Chief (Technical Division)	Outstanding Support for Research Award, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
MAKINO, Yumiko	Vice-Unit Chief (Technical Division)	
YAMAGUCHI, Katsushi	Vice-Unit Chief (Technical Division)	
BINO, Takahiro	Technical Staff (Technical Division)	
TOKUTSU, Ryutaro	Assistant Professor	The Young Scientists' Award, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
SOYANO, Takashi	Associate Professor	Journal of Plant Research Best Paper Award
FUJITA, Hironori	Assistant Professor	
HASHIMOTO, Kayo	Postdoctoral Fellow	
NAKAGAWA, Tomomi	Visiting Scientist	
KANAZAWA, Takehiko	Assistant Professor	BSJ Young Botanist Prize, The Botanical Society of Japan Awards
KIM, Eunchul	Assistant Professor	10th NINS Award for Young Scientists
ANDO, Toshiya	Assistant Professor	Encouraging Prize, The Zoological Society of Japan
NISHIUMI, Nozomi	JSPS Postdoctoral Fellow	Japan Ethological Society Award
FUKUDOME, Mitsutaka	JSPS Postdoctoral Fellow	Research Paper Award 2020 of The Microbes and Environments
MAEDA, Taro	Postdoctoral Fellow	
KAWAGUCHI, Masayoshi	Professor	
NAGAHAMA, Yoshitaka	Professor Emeritus	The Order of the Sacred Treasure, Gold Rays with Neck Ribbon
HOSHINO, Atsushi	Assistant Professor	93rd Best Papers Award, The Genetics Society of Japan
AGATA, Kiyokazu	Director General	Science Lectureship Award 2021, Chiba University

* Changes in professors, associate/ assistant professors are shown below.

Selected Publications in 2021AY from NIBB

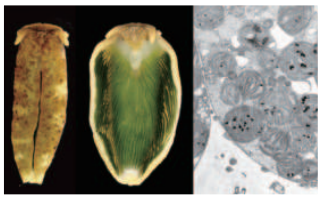
Transient suppression of transplanted spermatogonial stem cell differentiation restores fertility in mice

Nakamura, Y., Jörg, D. J., Kon, Y., Simons, B. D., and Yoshida, S. (2021). Cell Stem Cell, *28*, 1443-1456.e7.
DOI: 10.1016/j.stem.2021.03.016



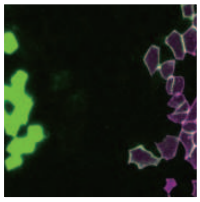
Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*

Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., Shigenobu, S. (2021). eLife *10*, e60176.
DOI: 10.7554/eLife.55108



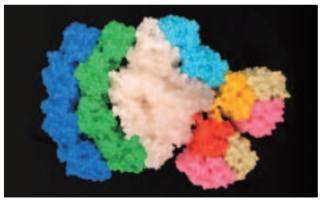
Quantitative analyses reveal extracellular dynamics of Wnt ligands in *Xenopus* embryos

Mii, Y., Nakazato, K., Pack, C. G., Ikeda, T., Sako, Y., Mochizuki, A., Taira, M. and Takada, S. (2021). eLife *10*, e55108.
DOI: 10.7554/eLife.60176



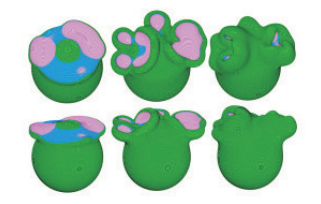
Structural basis of LhcbM5-mediated state transitions in green algae

Pan, X., Tokutsu, R., Li, A., Takizawa, K., Song, C., Murata, K., Yamasaki, T., Liu, Z., Minagawa, J., and Li, M. (2021). Nat. Plants *7*, 1119–1131.
DOI: 10.1038/s41477-021-00960-8



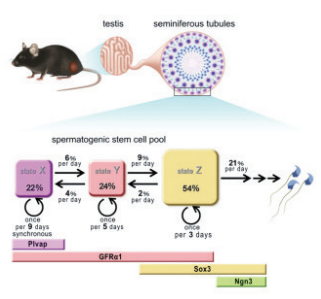
Three-dimensional morphodynamics simulations of macropinocytic cups

Saito, N., Sawai, S. (2021). iScience *24*, 103087.
DOI: 10.1016/j.isci.2021.103087



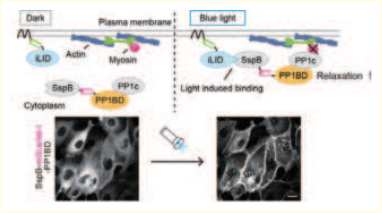
A multistate stem cell dynamics maintains homeostasis in mouse spermatogenesis

Nakagawa, T., Jörg, D. J., Watanabe, H., Mizuno, S., Han, S., Ikeda, T., Omatsu, Y., Nishimura, K., Fujita, M., Takahashi, S., Kondoh, G., Simons, B. D., Yoshida S. and Nagasawa, T. (2021). Cell Reports *37*, 109875.
DOI: 10.1016/j.celrep.2021.109875



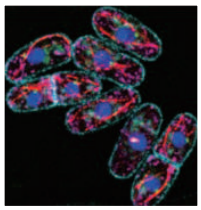
Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis

Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y. and Aoki, K. (2021). Nat. Commun. *12*, 7145.
DOI: 10.1038/s41467-021-27458-3



Near-infrared imaging in fission yeast using a genetically encoded phycocyanobilin biosynthesis system

Sakai, K., Kondo, Y., Fujioka, H., Kamiya, M., Aoki, K., and Goto, Y. (2021). J. Cell Sci. *134*, jcs259315.
DOI：10.1242/jcs.259315

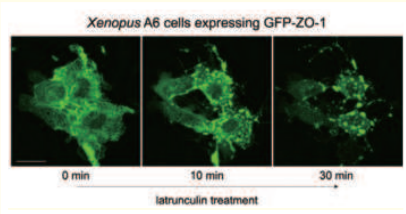


Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: gene duplication facilitates social evolution

Shigenobu, S., Hayashi, Y., Watanabe, D., Tokuda, G., Hojo, M.Y., Toga, K., Saiki, R., Yaguchi, H., Masuoka, Y., Suzuki, R., Suzuki, S., Kimura, M., Matsunami, M., Sugime, Y., Oguchi, K., Niimi, T., Gotoh, H., Hojo, M.K., Miyazaki, S., Toyoda, A., Miura, T., and Maekawa, K. (2022). Proc. Natl. Acad. Sci. U.S.A. *119*, e2110361119.
DOI: 10.1073/pnas.2110361119

Force-dependent remodeling of cytoplasmic ZO-1 condensates contributes to cell-cell adhesion through enhancing tight junctions

Kinoshita, N., Yamamoto, T.S., Yasue, N., Takagi, C., Fujimori, T., Ueno, N. (2022). iScience *25*, 103846.
DOI: 10.1016/j.isci.2022.103846

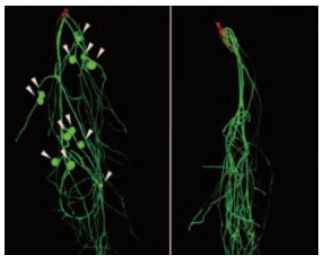


A hemimetabolous wing development suggests the wing origin from lateral tergum of a wingless ancestor

Ohde, T., Mito, T., and Niimi, T. (2022). Nat. Commun. *13*, 979.
DOI: 10.1038/s41467-022-28624-x

Auxin methylation by *IAMT1*, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus*

Goto, T., Soyano, T., Liu, M., Mori, T., Kawaguchi, M. (2022). Proc. Natl. Acad. Sci. U.S.A. *119*, e2116549119.
DOI: 10.1073/pnas.2116549119



Motion Illusion-like Patterns Extracted from Photo and Art Images Using Predictive Deep Neural Networks

Kobayashi, T., Kitaoka, A., Kosaka, M., Tanaka, K., and Watanabe, E. (2022). Sci. Rep. *12*, 3893.
DOI: 10.1038/s41598-022-07438-3

Professor Emeritus

Professor Emeritus
TAKEUCHI, Ikuo

Professor Emeritus
MOHRI, Hideo

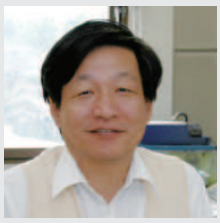
Professor Emeritus
KATSUKI, Motoya

Professor Emeritus
NAGAHAMA, Yoshitaka

Professor Emeritus
OHSUMI, Yoshinori

Professor Emeritus
HORIUCHI, Takashi

Professor Emeritus
OKADA, Kiyotaka



Professor Emeritus
NISHIMURA, Mikio

Professor Emeritus
YAMAMORI, Tetsuo

Professor Emeritus
IGUCHI, Taisen

Professor Emeritus
YAMAMOTO, Masayuki

Professor Emeritus
NODA, Masaharu

Publication List of Prof. NAGAHAMA, Yoshitaka

〔Original paper〕

- Murata, R., Nozu, R., Mushirobira, Y., Amagai, T., Fushimi, J., Kobayashi, Y., Soyano, K., Nagahama, Y., and Nakamura, M. (2021). Testicular inducing steroidogenic cells trigger sex change in groupers. Sci. Rep. *11*, 11117. DOI: 10.1038/s41598-021-90691-9

〔Review Article〕

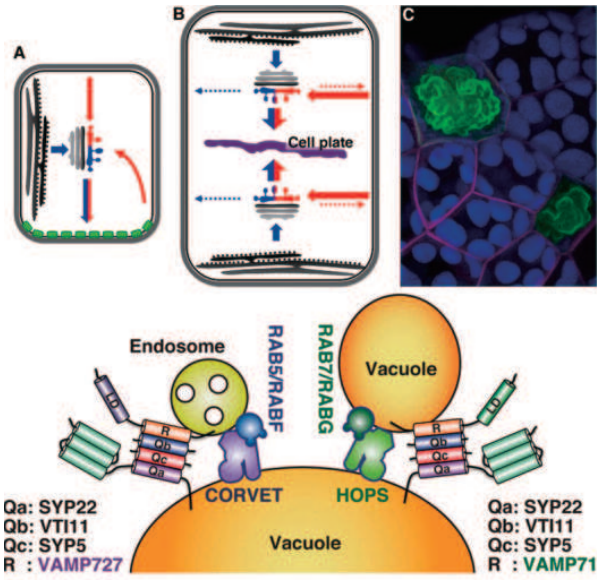
- Nagahama, Y., Chakraborty, T., Paul-Prasanth, B., Ohta, K., and Nakamura, M. (2021). Sex determination, gonadal sex differentiation, and plasticity in vertebrate species. Physiol. Rev. *101*, 1237–1308. DOI: 10.1152/physrev.00044.2019

DIVISION OF CELLULAR DYNAMICS

Professor
UEDA, Takashi

Assistant Professor:	*EBINE, Kazuo*
	KANAZAWA, Takehiko
Specially Appointed Assistant Professor:	*MINAMINO, Naoki*
Technical Staff:	*HAYASHI, Kohji*
Postdoctoral Fellow:	*TSUGANE, Mika*
	NORIZUKI, Takuya
	HIWATASHI, Takuma
	FENG, Yihong
SOKENDAI Graduate Student:	*HACHINODA, Sho*
Technical Assistant:	*YAMAMOTO, Mayuko*
	YOSHINORI, Yumi
	TANABE, Yoshimi
Admin Support Staff:	*OKUBO, Masayo*

Membrane traffic between single membrane-bounded organelles plays an integral role in various activities in eukaryotic cells. Recent comparative genomics has 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. In collaboration with other groups with expertise in artificial intelligence, we are also developing a framework to deliver new findings in the basic biology field.



Visual overview of this lab's work.

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 has 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 and specialization of membrane trafficking pathways in land plants have 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

The oil body is an organelle specific to liverworts, whose origin and biogenesis mechanism remained unclear for over a hundred years. We are studying the oil body in *M. polymorpha*, as a model of newly-acquired organelles in specific lineages during evolution. Through comprehensive analyses of SNARE members and organelle markers in *M. polymorpha*, we identified that a member of the SYP1 group, which generally acts at the plasma membrane in the secretory pathway, was targeted to the oil body. Together with other lines of evidence, we concluded that the oil body is formed by the redirection of the secretory pathway. We additionally proposed the oil body cycle hypothesis; the periodic redirection of the secretory pathway mediates oil body development and growth of the oil body cell. Furthermore, we revealed that the oil body acts as a defense against potential arthropod herbivory by using mutants of the master regulator of oil body formation that we identified, MpERF13.

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 with abnormally shaped oil bodies in *M. polymorpha*, a mutant of which harbors a mutation in a subunit of the COPI coat complex (Figure 1). Given that COPI acts around the Golgi apparatus and plays

an essential role in secretion, our finding further supports that the liverwort oil body is formed by the redirection of the secretory pathway.

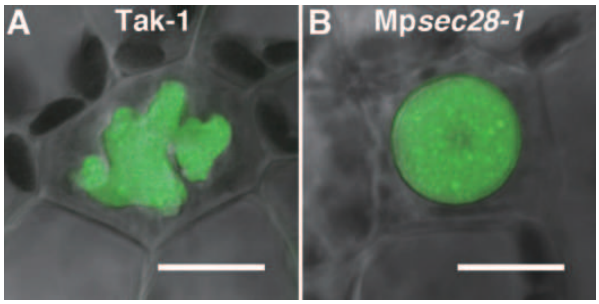


Figure 1. COPI-mediated secretory activity is required for normal oil body formation. The oil body is a complex and bumpy-surfaced structure in wild type (A). In the Mp*sec28-1* mutant, the oil body is more rounded with a smoother-surface (B). The oil bodies were visualized using BODIPY 493/503 (green). Bars = 10 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.

Plant-specific features of the vacuolar transport were partly achieved by acquisition of a plant-unique SNARE protein, VAMP727. VAMP727 executes membrane fusion between the multivesicular endosome and vacuole, assisted by the CORVET tethering complex. After execution of the membrane fusion, VAMP727 must be recycled back from the vacuolar membrane for sustainable vacuolar transport. However, mechanisms of recycling from the plant vacuolar membrane remain totally unknown. By using VAMP727 as a model cargo, we are currently exploring molecular mechanisms of recycling from the vacuolar membrane. We already succeeded in isolating several candidates that could act in this trafficking pathway.

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

2-1 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. The ANTH group has been remarkably expanded during land plant evolution, and we are investigating how this protein family has been functionally diversified in *A. thaliana*. We found that a pair of ANTH proteins, PICALM1a and PICALM1b, are required for retrieving a secretory SNARE protein, VAMP721, from the plasma membrane. This function is required for normal vegetative development of *A. thaliana* (Figure 2). This finding also highlighted the divergent mechanisms of VAMP7 recycling from the plasma membrane between plants and animals.

We also found that another paralogous set of PICALM proteins, PICALM5a and 5b is required for tip-localization of ANXUR receptor kinases acting in an autocrine signaling pathway required for pollen tube integrity in *A. thaliana* (Figure 2). Thus, functionally differentiated ANTH proteins underpin various physiological processes in *A. thaliana*.

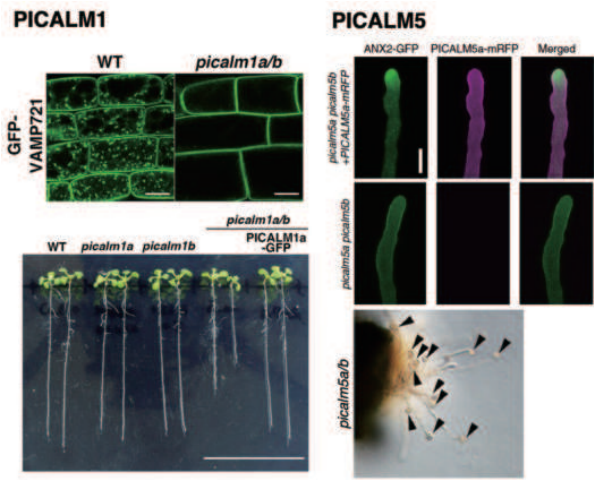


Figure 2. PICALM1 and PICALM5 mediate endocytosis of distinct plasma membrane proteins, which are required for normal vegetative development and pollen tube integrity, respectively. (Left: Fujimoto *et al*., 2020, modified, right: Muro *et al*., 2018, modified)

In addition to PICALM5, PICALM2, PICALM6, and PICALM9 are also highly expressed in *A. thaliana* pollen and/or pollen tubes, which suggests that these PICALMs underpin the complex endocytosis system in pollen tubes. Recently, we found that the *picalm2a/2b* double mutant exhibits defective transmission of the mutation from male plants, which suggested that PICALM2 acts in endocytic transport of important but still unknown cargo(s) in pollen and/or pollen tubes. Now we are investigating molecular mechanisms of endocytosis in pollen tubes with a special focus on clathrin adaptor proteins including PICALM members.

Our results indicate that each PICALM protein regulates endocytosis of distinctive cargo proteins, whose molecular mechanisms remain mostly unclear in plants. We are now investigating the molecular function of PICALMs with a special focus on the mechanisms of cargo recognition by PICALMs in *A. thaliana*.

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 an important role in membrane fusion in the formation 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*.

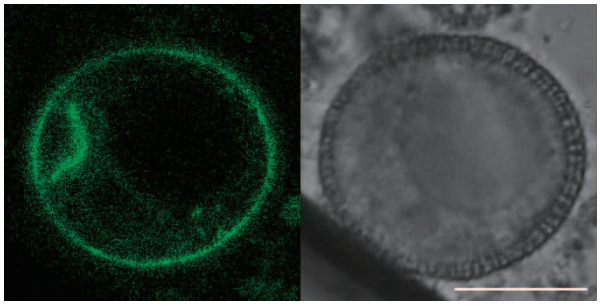


Figure 3. Expression and subcellular localization of GFP-KNOLLE during pollen mitosis I. GFP-KNOLLE accumulates at the cell plate. Bar = 10 μm.

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, is 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.

Autophagy-defective mutants exhibited defects regarding cytoplasm removal, spermatozoid motility, and fertility. Although a majority of organelles are removed during spermiogenesis, a specific set of organelles, *e.g*. anterior and posterior mitochondria, persists in mature spermatozoids (Figure 4), which implies that there should be a mechanism for selective removal of unneeded organelles. By analyzing the effect of defective autophagy during spermiogenesis in *M. polymorpha*, we found that spermiogenesis occurs through multimodal autophagic degradation.

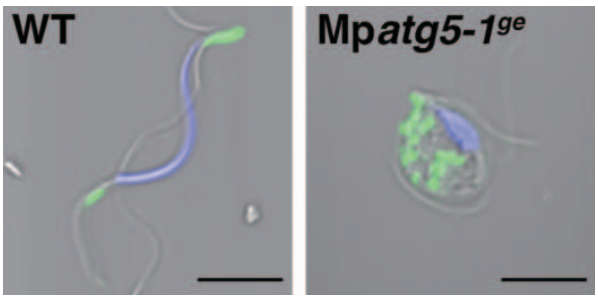


Figure 4. Impaired reorganization of mitochondria in an autophagy-defective mutant of *M. polymorpha*. The wild-type spermatozoid possesses two mitochondria (green), whereas a number of mitochondria remained in the Mp*atg5-1*^{ge} mutant spermatozoid. Blue: nuclei. Bars = 5 μm.

We also found that spermiogenesis requires the proper function of the ESCRTs complex, which mediates multivesicular endosome formation and sorting of membrane proteins to be degraded. These results indicate that highly organized autophagic and non-autophagic degradation play crucial roles to accomplish spermiogenesis in *M. polymorpha.*

We are also analyzing the role of RAB GTPases in flagella formation. Through a comprehensive analysis of RAB GTPases in *M. polymorpha*, we found that a RAB GTPase plays an essential role in generating fully functional flagella (Figure 5).

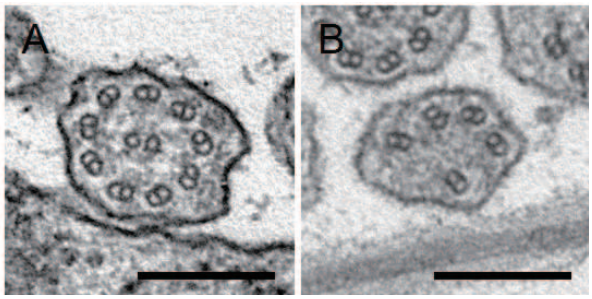


Figure 5. Transverse sections of flagella in wild-type (A) and mutant (B) spermatids. The microtubule-based "9 + 2" axoneme structure is severely compromised in the mutant. Bars = 200 nm.

Publication List:

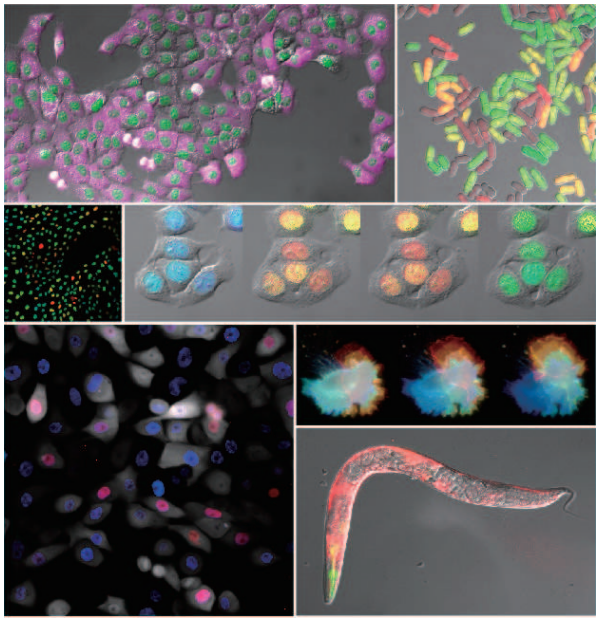
〔Original paper〕

- Shimada, T.L., Ueda, T., and Hara-Nishimura, I. (2021). Excess sterol accumulation affects seed morphology and physiology in *Arabidopsis thaliana*. Plant Signal. Behav. *16*, 1872217. DOI: 10.1080/15592324.2021.1872217

DIVISION OF QUANTITATIVE BIOLOGY

Professor
AOKI, Kazuhiro

Assistant Professor:	*KONDO, Yohei*
	GOTO, Yuhei
Technical Staff:	*BINO, Takahiro*
Postdoctoral Fellow:	*NAKAMURA, Akinobu*
JSPS Postdoctoral Fellow:	*SUGIYAMA, Hironori*
Research Staff:	*EBINE, Emi*
	TANII, Ryosuke
	GOTO, Yoko
SOKENDAI Graduate Student:	*MUKAI, Masaya*
	YAMAMOTO, Kei
	SAKAI, Keiichiro
	TSURUOKA, Tatsuki
	ITOH, Thoma
Visiting Scientist:	*ITO, Reina*
Admin Support Staff:	*ONODA, Kaori*



Visual overview of this lab's work, showing fluorescence images of mammalian cells, fission yeast, and *C.elegans*

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 implemented by an intracellular signal transduction system, which is comprised of a series of chemical reactions such as protein-protein interactions and protein phosphorylation. Dysregulation of cell signaling by gene mutation is widely known to result in various pathologies, such as malignant tumors.

The intracellular signal transduction system has been extensively studied over the past few decades through approaches utilizing both biochemistry and cell biology approaches. As a result, many proteins and regulations have been identified, which has resulted in an increase in the pathway's complexity. The complicated signaling network makes it difficult to understand how cells process information and quantitatively make decisions.

To address these issues, we are currently focusing on the development of two types of research tools that enable us to (1) visualize and (2) manipulate intracellular signaling pathways (Figure 1).

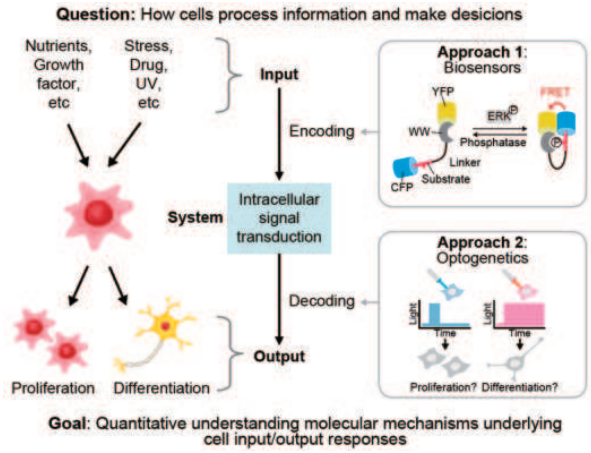


Figure 1. Information processing by intracellular signaling devices and networks.

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. FRET-based biosensors allow us to detect PKA, ERK, Akt, JNK, PKC, and S6K's kinase activity in living cells at a high temporal and spatial resolution (Komatsu N, Mol. Biol. Cell, 2011; Aoki K, Mol Cell, 2013; Aoki K, Dev Cell, 2017).

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, and to monitor it in living cells and animals, we have designed a red-fluorescent DA reporter. This reporter features a circular-permutated mApple (cpmApple), a red fluorescent protein, and a 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 (Figures 2B and 2C). In addition, by using a previously reported green fluorescence norepinephrine (NE) reporter, we succeeded in simultaneously visualizing DA and NE in living neurons (Nakamoto C, Goto Y, Mol Brain, 2021).

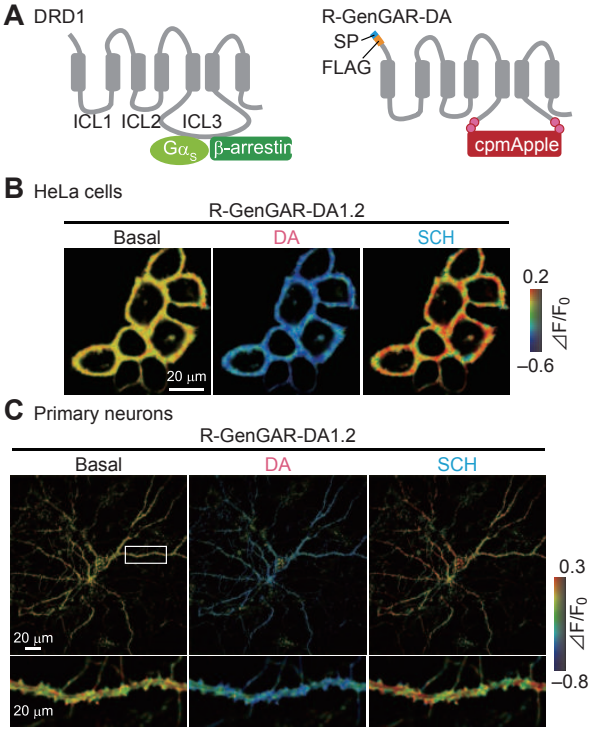


Figure 2. Development of a genetically encoded red fluorescent dopamine (DA) reporter. (A) Schematic representation of a dopamine receptor, DRD1 (left), and Red DA reporter, R-GenGAR-DA. (B and C) HeLa cells (B) or mouse primary hippocampal neurons (C) expressing R-GenGAR-DA were stimulated with DA,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

Near-infrared fluorescent protein (iRFP) is a fluorescent protein with near-infrared excitation and emission maxima. Unlike the other conventional fluorescent proteins, iRFP requires biliverdin (BV) as a chromophore (Figure 3A). We accidentally found that phycocyanobilin (PCB) functions as a brighter chromophore for iRFP than BV and that biosynthesis of PCB allows live-cell imaging with iRFP in the fission yeast *Schizosaccharomyces pombe* (Figure 3B). We initially found that fission yeast cells did not produce BV and therefore did not show any iRFP fluorescence (Figure 3C). The brightness of iRFP–PCB was higher than that of iRFP–BV both *in vitro* and in fission yeast. We introduced SynPCB2.1, a PCB biosynthesis system (Uda, Miura, ACS Chem Biol, 2020), into fission yeast, resulting in the brightest iRFP fluorescence. These tools not only enable the easy use of multiplexed live-cell imaging in fission yeast with a broader color palette, but also open the door to new opportunities for near-infrared fluorescence imaging in a broader range of living organisms (Sakai, JCS, 2021).

II. Manipulation of cell signaling

Artificial manipulation of biochemical networks is well established in the quantitative understanding of biological systems. Multiple methods have been suggested for control-

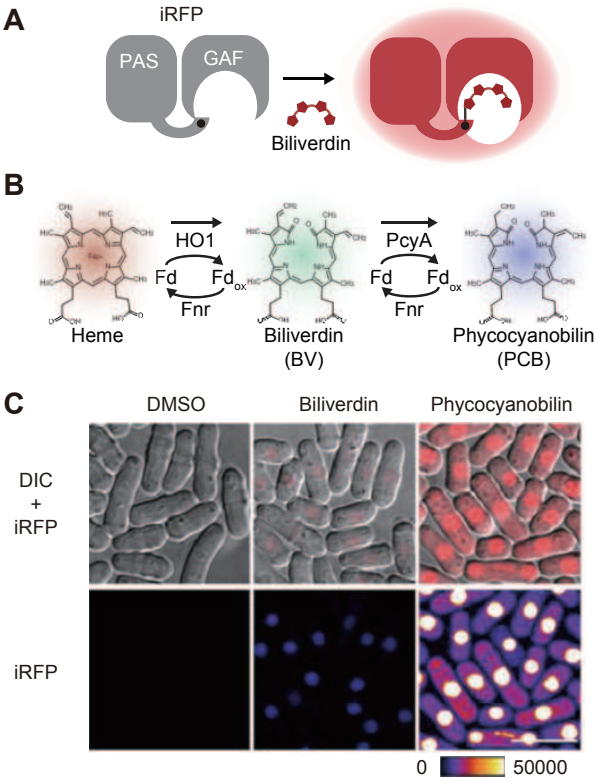


Figure 3. Phycocyanobilin (PCB) as a brighter chromophore for iRFP. (A) Schematic representation of iRFP with its chromphore Bliverdin. (B) The metabolic pathway of PCB. (C) DIC and iRFP fluorescence images are shown in fission yeats treated with DMSO, Biliverdin, or PCB.

ling signal transduction, including light-induced dimerization (LID) and chemically induced dimerization (CID) systems. Of these two, the LID system is more beneficial in terms of temporal and spatial manipulation. The photo-responsive proteins derived from fungi, cyanobacteria, plants, and modified fluorescent proteins are used in this system.

We have reported a novel optogenetic tool to induce relaxation of actomyosin contractility with a blue light-responsive protein, such as cryptochrome 2 (CRY2) and iLID. Actomyosin contractility, which is generated cooperatively by nonmuscle myosin II (NMII) and actin filaments, plays essential roles in a wide range of biological processes such as cell motility and cytokinesis. Yet, it remains elusive as to how actomyosin contractility generates force and maintains cellular morphology. To elucidate this, we have developed a novel optogenetic tool, OptoMYPT, which combines a catalytic subunit of type I phosphatase-binding domain of MYPT1 with an optogenetic dimerizer so that it allows light-dependent recruitment of endogenous PP1c to the plasma membrane (Figure 4A). We confirmed that blue-light illumination was sufficient to induce dephosphorylation of MLC, resulting in lamellipodial membrane protrusion (Figure 4B). The OptoMYPT was further employed to understand the mechanics of actomyosin-based cortical tension and contractile ring tension during cytokinesis (Figure 4C). We found that the relaxation of cortical tension at both poles by OptoMYPT accelerated the furrow ingression rate, thus revealing that the cortical tension substantially antagonizes constriction of the cleavage furrow. These results provide new opportunities to freely design cell and tissue morphology through light (Yamamoto, Nat Comm, 2021).

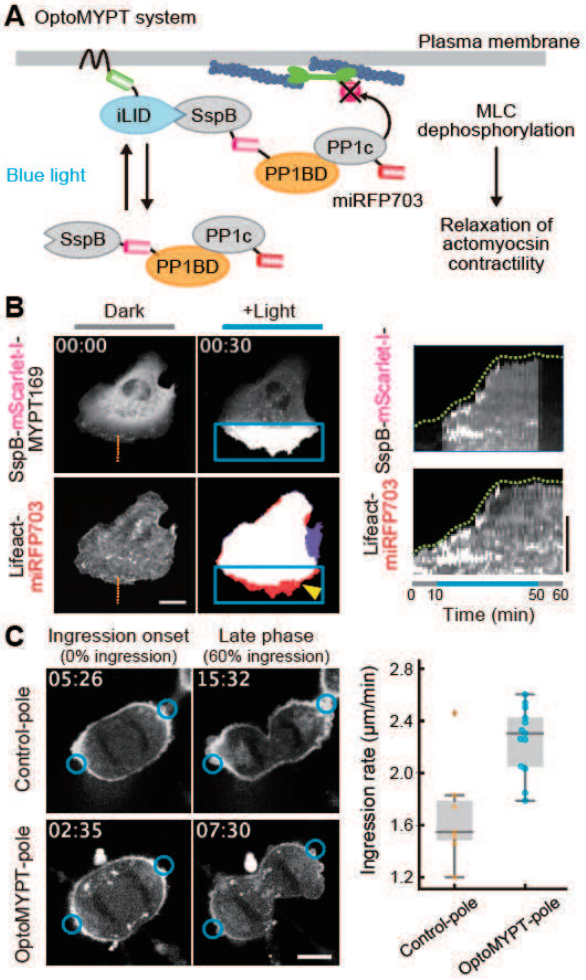


Figure 4. PhyB-PIF light-inducible dimerization (LID) system. (A) The metabolic pathway of phytochrome chromophores, PCB. (B and C) Light-induced translocation of PIF3-mEGFP to the plasma membrane, where PhyB is localized (B). Membrane translocation of PIF3-mEGFP is quantified (C).

The Blue light-responsive optical dimerizers are useful, but its activation light often overlaps with the excitation light of fluorescent proteins, which in turn hampers the application of GFP or FRET biosensors together with their optogenetic tools. To circumvent this issue, 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. One drawback of PhyB-PIF LID is that a chromophore, *e.g.*, PCB, is required for the light sensing. To overcome this issue, we have developed a method for PCB biosynthesis, called SynPCB, in mammalian cells by introducing the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into the mitochondria (Uda Y, PNAS, 2017; Uda Y, ACS Chem Biol, 2020).

To take full advantage of this system, we applied the SynPCB 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* 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, and Opto-SAC induced a spindle assembly checkpoint (SAC) in response to red light (Figure 5) (Goto Y, and Aoki K., bioRxiv, 2020).

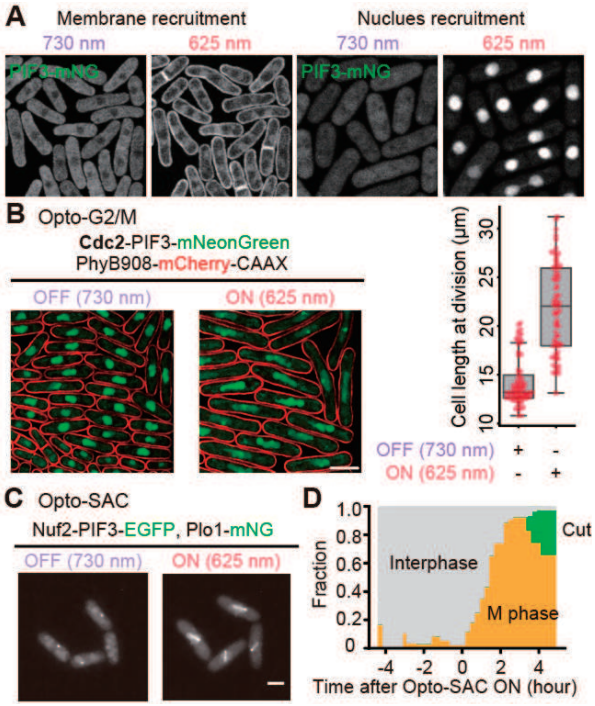


Figure 5. Optogenetic control of cell cycle in fission yeast. (A) Light-induced recruitment of PIF3-mNeonGreen (mNG) to the plasma membrane (left) and nucleus (right). (B) Opto-G2/M: Optical control of G2/M transition. (left) Opto-G2/M OFF cells showed normal morphology, while Opto-G2/M ON cells exhibited longer cell length, a typical phenotype of G2/M arrest fission yeast. (C) Opto-SAC: Optical control of spindle assembly checkpoint. Representative cells undergoing metaphase arrest (right) by Opto-SAC. (D) Long-term metaphase arrest by Opto-SAC leads to a mitotic failure and cut phenotype.

Publication List:

〔Original papers〕

- Aragaki, H., Ogoh, K., Kondo, Y., and Aoki, K. (2022). LIM Tracker: a software package for cell tracking and analysis with advanced interactivity. Sci. Rep. *12*, 2702. DOI: 10.1038/s41598-022-06269-6
- Ito, R.E., Oneyama, C., and Aoki, K. (2021). Oncogenic mutation or overexpression of oncogenic KRAS or BRAF is not sufficient to confer oncogene addiction. PLoS One *16*, e0249388. DOI: 10.1371/journal.pone.0249388
- Kajiwara, K., Yamano, S., Aoki, K., Okuzaki, D., Matsumoto, K., and Okada, M. (2021). CDCP1 promotes compensatory renal growth by integrating Src and Met signaling. Life Sci. Alliance *4*, e202000832. DOI: 10.26508/lsa.202000832
- Lin, S., Hirayama, D., Maryu, G., Matsuda, K., Hino, N., Deguchi, E., Aoki, K., Iwamoto, R., Terai, K., and Matsuda, M. (2022). Redundant roles of EGFR ligands in the ERK activation waves during collective cell migration. Life Sci. Alliance *5*, e202101206. DOI: 10.26508/lsa.202101206
- Matsui, K., Okamoto, K., Hasegawa, T., Ohtsuka, H., Shimasaki, T., Ihara, K., Goto, Y., Aoki, K., and Aiba, H. (2021). Identification of ksg1 mutation showing long-lived phenotype in fission yeast. Genes to Cells *26*, 967–978. DOI: 10.1111/gtc.12897
- Nakamoto, C., Goto, Y., Tomizawa, Y., Fukata, Y., Fukata, M., Harpsoe, K., Gloriam, D.E., Aoki, K., and Takeuchi, T. (2021). A novel red fluorescence dopamine biosensor selectively detects dopamine in the presence of norepinephrine in vitro. Mol. Brain *14*, 173. DOI: 10.1186/s13041-021-00882-8
- Nakayama, H., Rowland, S.D., Cheng, Z., Zumstein, K., Kang, J., Kondo, Y., and Sinha, N.R. (2021). Leaf form diversification in an ornamental heirloom tomato results from alterations in two different HOMEOBOX genes. Curr. Biol. *31*, 4788+. DOI: 10.1016/j.cub.2021.08.023
- Sakai, K., Kondo, Y., Fujioka, H., Kamiya, M., Aoki, K., and Goto,

Y. (2021). Near-infrared imaging in fission yeast using a genetically encoded phycocyanobilin biosynthesis system. J. Cell Sci. *134*, jcs259315. DOI: 10.1242/jcs.259315

- Verma, A., Jena, S.G., Isakov, D.R., Aoki, K., Toettcher, J.E., and Engelhardt, B.E. (2021). A self-exciting point process to study multicellular spatial signaling patterns. Proc. Natl. Acad. Sci. U.S.A. *118*. e2026123118. DOI: 10.1073/pnas.2026123118
- Yagi-Utsumi, M., Aoki, K., Watanabe, H., Song, C., Nishimura, S., Satoh, T., Yanaka, S., Ganser, C., Tanaka, S., Schnapka, V., Goh, E.W., Furutani, Y., Murata, K., Uchihashi, T., Arakawa, K., and Kato, K. (2021). Desiccation-induced fibrous condensation of CAHS protein from an anhydrobiotic tardigrade. Sci. Rep. *11*. 21328. DOI: 10.1038/s41598-021-00724-6
- Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y., and Aoki, K. (2021). Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. Nat. Commun. *12*. 7145. DOI: 10.1038/s41467-021-27458-3

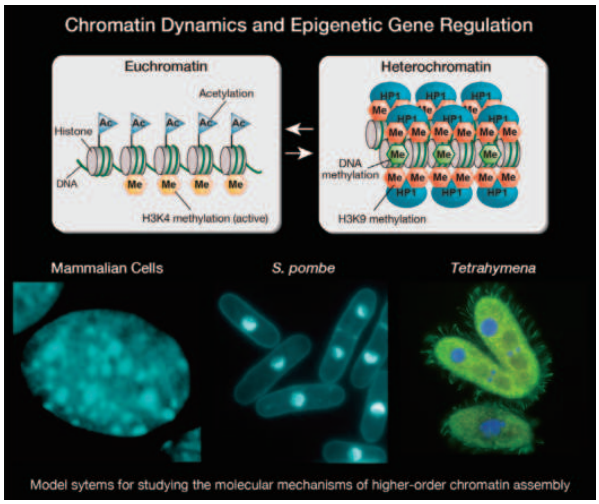
〔Review Articles〕

- Farahani, P.E., Reed, E.H., Underhill, E.J., Aoki, K., and Toettcher, J.E. (2021). Signaling, Deconstructed: Using Optogenetics to Dissect and Direct Information Flow in Biological Systems. Annu. Rev. Biomed. Eng. *23*, 61–87. DOI: 10.1146/annurev-bioeng-083120-111648
- Hiiragi, T., Gladfelter, A.S., Miguel-Aliaga, I., Coller, H.A., Lau, O.S., McBride, H.M., Aoki, K., Linkermann, A., Santaguida, S., Wasteneys, G., Rivron, N.C., Shahbazi, M.N., Ebisuya, M., and Lancaster, M.A. (2021). 20 years of Developmental Cell: Looking forward. Dev. Cell *56*, 3185–3191. DOI: 10.1016/j.devcel.2021.11.017
- Nakamura, A., Goto, Y., Kondo, Y., and Aoki, K. (2021). Shedding light on developmental ERK signaling with genetically encoded biosensors. Development *148*. dev199767. DOI: 10.1242/dev.199767

DIVISION OF CHROMATIN REGULATION

Professor
NAKAYAMA, Jun-ichi

Assistant Professor: *KATAOKA, Kensuke*
KAWAGUCHI, Takayuki
Specially Appointed Assistant Professor:
HAYASHI, Aki
Technical Staff: *NISHIMOTO, Yuki*
SOKENDAI Graduate Student: *RAHAYU, Anisa*
VALENTIROVIC, Olivera
NAKAMURA, Rinko
YOSHIDA, Takaki
OYA, Tomoyuki
Visiting Scientist: *HAMADA, Kyoko*
Technical Assistant: *YOSHIMURA, Yuriko*
ASAI, Yuriko
Admin Support Staff: *KIYOHARA, Megumi*



Model sytems for studying the molecular mechanisms of higher-order chromatin assembly

The stable inheritance of gene expression or repression states is essential to cellular differentiation and development, and an epigenetic cellular-memory system derived from higher-order chromatin structure plays a fundamental role in this process. The assembly of higher-order chromatin structures has been linked to the covalent modifications of histone tails, chromatin-associated proteins, and non-coding RNAs. However, the exact means by which this 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 model systems including mammalian culture cells, fission yeast, *Schizosaccharomyces pombe* (*S. pombe*), and ciliate *Tetrahymena* for studying the assembly of higher-order chromatin structures, known as heterochromatin. 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 Mechanisms regulating Clr4 histone methyltransferase activity

In eukaryotic cells, the assembly of heterochromatin plays an important role in diverse chromosomal processes and epigenetic gene regulation. Heterochromatin is characterized by the methylation of histone H3 at lysine 9 (H3K9me). H3K9me is catalyzed by SUV39H-family histone methyltransferases, and functions as a binding site for recruiting heterochromatin protein 1 (HP1) family proteins. In fission yeast, H3K9me is catalyzed by histone methyltransferase Clr4. Clr4 has two functional domains, the N-terminal chromodomain (CD), which recognizes H3K9me, and the C-terminal SET domain responsible for Clr4's enzymatic activity (Figure 1A). Since Clr4's uncontrolled activity leads to inappropriate H3K9me and aberrant gene silencing, its enzymatic activity needs to be strictly controlled. However, the detailed mechanisms regulating Clr4's activity are poorly understood.

A previous study showed that N-terminally deleted Clr4 mutant exhibits a stronger activity than full-length Clr4, suggesting that the Clr4 N-terminal region negatively regulates the activity of C-terminal SET domain. To examine the regulation mechanisms of Clr4's activity, we tested whether Clr4 N-terminal region interacts with its C-terminal SET domain. Pull-down assays using recombinant proteins reveal that the Clr4 N-terminal region physically interacts with the SET domain and that this interaction requires both CD and its adjacent region. To determine the regions/residues responsible for this interaction, we performed crosslinking

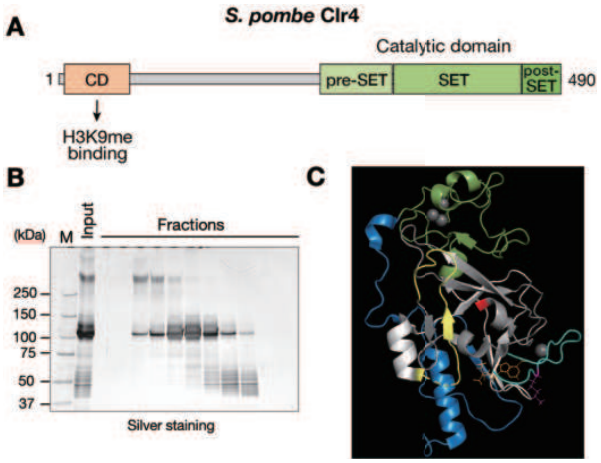


Figure 1. Characterization of intramolecular interactions of Clr4. (A) Domain organization of Clr4. The N-terminal chromodomain (CD) recognizes H3K9me, and the C-terminal SET domain is responsible for Clr4's enzymatic activity. (B) Crosslinking mass spectrometry. After *in vitro* crosslinking reaction, recombinant Clr4 proteins were separated by gel-filtration chromatography and analyzed by SDS-PAGE and silver staining. (C) Residues identified in the cross-linking mass spectrometry to be involved in the interaction are mapped on 3D structure of Clr4 SET domain.

mass spectrometry (Figure 1B) and found that crosslinked residues were concentrated in a region near the C-terminal end (Figure 1C). Interestingly, mutant Clr4 proteins containing amino-acid substitutions at this region exhibit higher methyltransferase activity. These results suggest that Clr4's enzymatic activity is negatively regulated by intramolecular interactions.

II. Roles of HP1 family proteins in assembling higher-order chromatin structure

2-1 Characterizing the role Chp2 in heterochromatin assembly in fission yeast

Heterochromatin protein 1 (HP1) is an evolutionarily conserved chromosomal protein that binds H3K9me, and plays a crucial role in forming higher-order chromatin structure. HP1 family proteins share a basic structure consisting of an N-terminal CD and a C-terminal chromoshadow domain (CSD) linked by an unstructured henge region (Figure 2A). The CD functions as a binding module that target H3K9me, whereas CSD functions as a dimerization module that provides a recognition surface to bind and recruit other proteins. In fission yeast, two HP1 family proteins, Swi6 and Chp2, are involved in heterochromatic silencing. While Swi6 is expressed abundantly and plays a dose-dependent role, Chp2 is expressed at a lower level and displays a novel ability to tightly bind chromatin-enriched fraction independently of H3K9me. To dissect how this Chp2-specific ability contributes to its function, we performed Tandem Affinity Purification (TAP) to identify Chp2-specific binding partners and successfully identified novel candidates for Chp2 binding partners. Furthermore, we examined Chp2-specific DNA-binding activity by Electrophoretic Mobility Shift Assay (EMSA) and found that both the hinge and CSD neighboring regions make distinct contribution to its DNA binding. These results suggest that both the Chp2's property to bind specific binding partners and its intrinsic DNA binding ability contribute to its tight chromatin association and also to division of labor between Swi6 and Chp2.

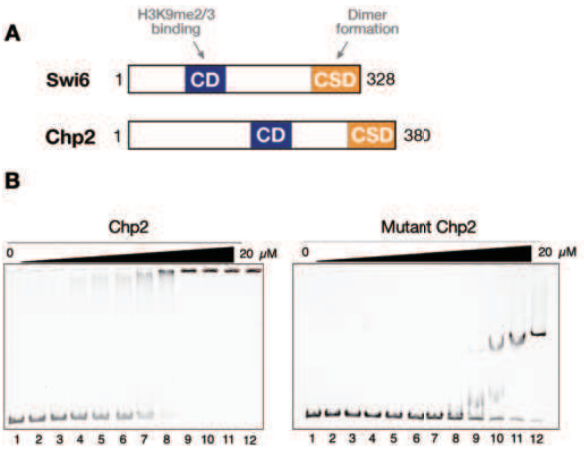


Figure 2. Chp2 possesses a strong DNA/RNA-binding activity. (A) Schematic diagram of Swi6 and Chp2. The chromodomain (CD) and the chromoshadow domains (CSD) are shown. (B) Representative results of EMSAs performed with control and mutant Chp2.

2-2 Multiple HP1-like protein-containing complex regulates DNA elimination in *Tetrahymena*

Heterochromatin plays important roles in transposon (TE) silencing. A major type of heterochromatin contains chromatin, that is histone H3 methylated at lysine 9/27 (H3K9/27me), and its reader HP1 proteins, that recruit diverse proteins onto the chromatin to silence the TEs. Although multiple HP1 proteins are co-expressed in many eukaryotic cells, the interplay between these HP1 proteins has been elusive. Here, we show that subset of the HP1 proteins form a complex and play important roles to eliminate TE-related Internal Eliminated Sequences (IESs) from the somatic genome during macronuclear development in the ciliated protozoan *Tetrahymena*. We tethered 7 HP1-like proteins individually to the artificially created locus by the LexA-LexO system and found that only 4 of them including Pdd1 induced the elimination of the tethered site. This ectopic DNA elimination was exclusively achieved by their chromoshadow domains (CSDs), indicating that the CSDs of the distinct type of HP1-like proteins recruits all the proteins that are required for DNA elimination. Immunoprecipitation of Pdd1 specifically enriched the other HP1-like proteins that were sufficient for the ectopic DNA elimination. The chromodomains of a subset of HP1-like proteins showed strong affinity to both H3K9me3 and H3K27me3. Overall, these results suggest that multiple HP1-like proteins cooperatively recognize the methylated histones and form core complex to recruit other effector proteins for DNA elimination (Figure 3).

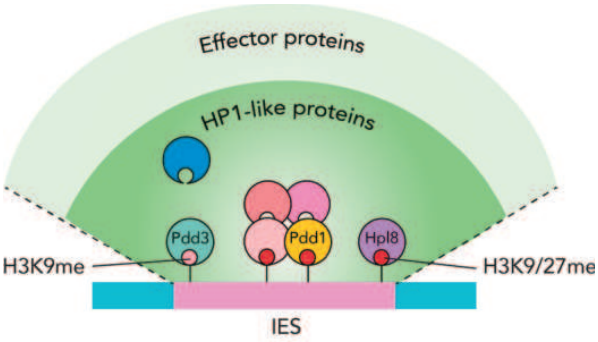


Figure 3. Model for how multiple HP1-like protein-containing complex regulates DNA elimination in *Tetrahymena*.

2-3 The HP1-like protein Hpl8p is important for programmed DNA elimination in *Tetrahymena*

Transposon mobility represents a threat for the genome integrity in all living things. To counter this, the ciliated protozoan *Tetrahymena* uses heterochromatin to selectively eliminate over 10,000 transposon loci from its somatic nucleus in the developmentally programmed process of DNA elimination. To address how this process is coordinated by heterochromatin components, we focus on an HP1-like protein named Hpl8p that localizes to the developing macronucleus during heterochromatin formation and DNA elimination (Figure 4). The chromodomain of Hpl8p recognized both H3K9me3 and H3K27me3 *in vitro*. *HPL8* disrupted mutants showed defect in vegetative growth and in the progression of sexual reproduction. FISH analyses using a probe against one of the transposons showed that more than

90% of *HPL8* disrupted cells did not complete its elimination, suggesting that Hpl8p plays important roles in DNA elimination. Altogether, these results suggest that Hpl8p binds the H3K9/27 methylation marks over transposon loci and facilitates the heterochromatin formation by interacting with heterochromatin components required for DNA elimination. Furthermore, we propose that Hpl8p also plays important roles in suppression of unwanted genes during the vegetative growth and sexual reproduction process possibly by recognizing H3K27me3 in the transcriptionally active macronucleus.

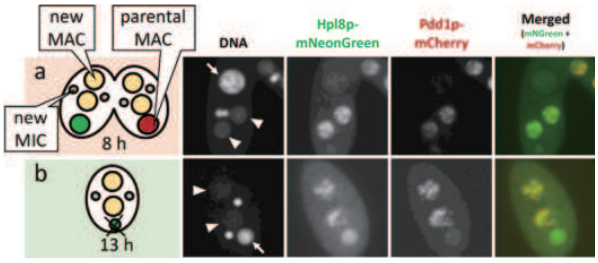


Figure 4. Cells expressing the Hpl8-mNeonGreen (green) and Pdd1p-mCherry (red) at 8 and 13 hours post induction of mating are shown in (a) and (b), respectively. DNA was counterstained with DAPI. Arrows and arrowheads indicate the parental and new MACs, respectively.

III. Analysis of chromatin dynamics in spore nuclei of fission yeast

Gametogenesis is a crucial process for sexually reproducing organisms to produce haploid gametes from diploid cells. Generally, epigenetic memory is erased from zygote nucleus, a phenomenon known as reprogramming, resulting in increased totipotency. On the other hand, it has been demonstrated in some organisms that a part of epigenetic memory can be inherited transgenerationally. The balance between reprogramming and epigenetic inheritance plays a key role in the developmental processes of offspring. It has been also reported that some gametes, like plant sperm and mammalian spermatozoa, have highly compacted nuclei and have altered histone modifications. However, the mechanisms underlying how epigenetic marks are regulated during gametogenesis remain to be fully elucidated. To gain an insight into epigenetic inheritance and chromosome structural change during gamete formation, we analyzed chromatin dynamics in spore nucleus of fission yeast (Figure 5A). We isolated fission yeast spores by density-gradient centrifugation and performed mass spectrometry analysis to determine spore-specific nuclear proteins (Figure 5B). We further examined the change in histone modifications of spore nuclei. Chromatin immunoprecipitation analysis revealed that the levels of H3K9me at heterochromatic regions had not been noticeably changed, but the levels of H3K4me associated transcriptionally active regions had clearly increased in spore nuclei. We also found that H3K4me distribution at coding regions in spore genome clearly different from that of control vegetative cells. These results imply that dynamic changes in active histone marks contribute to spore formation and epigenetic inheritance.

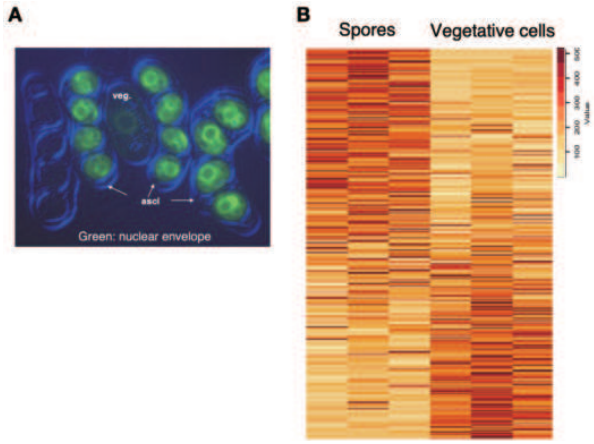


Figure 5. Analysis of chromatin dynamics in spore nuclei of fission yeast. (A) A representative image of vegetative cells (veg.) and spores in asci. (B) Heatmap representation of proteins detected in spores and vegetative cells.

Publication List:

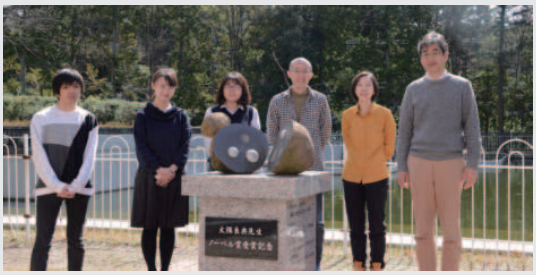
〔Original paper〕

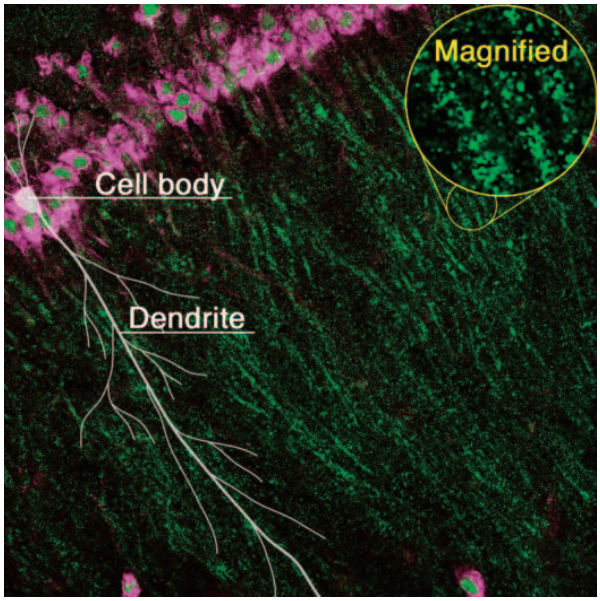
● Nakamura, R., and Nakayama, J. (2022). Multiple interfaces to recognize nucleosomal targets. J. Biochem. *171*, 257–259. DOI: 10.1093/jb/mvab139

LABORATORY OF NEURONAL CELL BIOLOGY

Associate Professor
SHIINA, Nobuyuki

Assistant Professor: *OHASHI, Rie*
SOKENDAI Graduate Student: *YAMASHITA, Akira*
HORIO, Tomoyo
ISHIKURA, Yui
YOSHIDA, Sho





RNA granules (green) transported from the cell body (magenta) to dendrites in the hippocampus of the mouse brain.

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 stably connect to each other, thereby forming long-term neural networks and memory. 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 membraneless organelles formed by liquid-liquid phase separation (LLPS) of RNA-binding proteins and mRNAs, which further recruit other factors such as ribosomes. They mediate the transport of mRNAs to the vicinity of synapses and synaptic stimulation-dependent local translation of the cargo mRNAs (Figure 1). We are currently using mice to research the mechanism of RNA granule assembly, RNA granule factors and their phase behavior regulating mRNA transport and local translation, their target mRNAs, and the roles of the locally synthesized proteins, so we can attain a better understanding of their relationship to the formation of synapses and neural networks, memory, learning, and behavior.

In addition to the physiological function, RNA granules are linked to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The basis of these diseases is thought to be the accumulation and aggregation of disease-causing proteins such as FUS and TDP-43 in RNA granules. Thus, our research also aims to reveal the effects of such protein aggregation on mRNA transport and local translation.

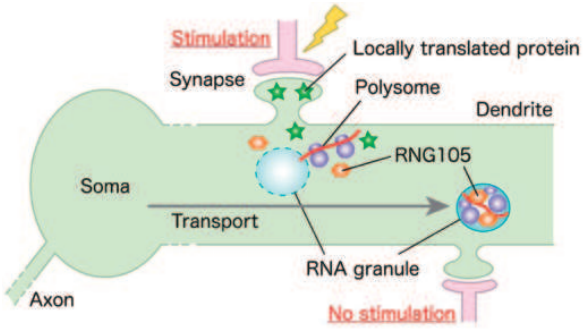


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Prion-like domain of ILF3 regulates gene expression and fear memory under chronic stress

LLPS is generally driven by protein intrinsically disordered regions (IDRs) that do not form three-dimensional structures. One class of IDR is the prion-like domain (PrLD), whose propensity to form aggregates has been extensively studied in the context of neurodegenerative diseases. However, little is known about the physiological relevance of PrLDs in the brain.

We focused on an RNA-binding protein NFAR2, which has PrLD at the C-terminus. This PrLD is spliced out in the alternative splicing isoform NFAR1. NFAR1 and NFAR2 are synthesized from the *Ilf3* gene, and both possess biochemical activities that they perform in common such as transcriptional regulation and stress-dependent translational inhibition. However, they differ in that NFAR2 alone can concentrate in the nucleoplasm and cytoplasmic RNA granules through PrLD. To investigate the physiological relevance of PrLD, we generated NFAR2ΔPrLD mice, in which a stop codon was introduced into the NFAR2-specific exon. This is considered an ideal strategy for revealing the physiological role specific to PrLD, as the splicing isoform without PrLD is intrinsically present and maintained even after PrLD deletion.

Deletion of the PrLD lost the nucleoplasmic localization of NFAR1/2 and affected the genome-wide profiles of

mRNA expression and translation in the brain. In particular, PrLD deletion significantly altered chronic restraint stress (CRS)-induced changes in mRNA expression and translation in the amygdala, a brain region associated with emotional events such as anxiety and fear.

Consistent with the effects on CRS-induced changes in mRNA expression and translation, PrLD deletion affected tolerance of mice to CRS in amygdala-associated learning and memory: conditioned fear memory in wild-type mice was unaffected by CRS, but it was exacerbated in NFAR2ΔPrLD mice due to CRS. These results indicated that PrLD of NFAR2 is responsible for the regulation of stress-responsive gene expression and confers stress tolerance in fear-associated memory formation, providing insights into PrLD's involvement in chronic stress adaptation.

II. Novel regulation of RNA granule dynamics by pathogenic accumulation of FUS and TDP-43 in RNA granules

FUS and TDP-43 are predominantly localized in the nucleus when cells are healthy, but in neurodegenerative diseases, they translocate to the cytoplasm and accumulate in RNA granules. FUS and TDP-43 prone to aggregate in RNA granules, which is thought to harden RNA granules, affect the function of RNA granules, and impair synapse formation and function. However, little is known about the effects of FUS and TDP-43 on the dynamics of other components of RNA granules.

We overexpressed disease-related FUS and TDP-43 mutants in primary cultured neurons from the mouse cerebral cortex and analyzed their effects on the dynamics of RNA granule components using fluorescence recovery after photobleaching (FRAP) and cell permeabilization assays (Shiina, *J. Biol. Chem.*, 2019). FUS and TDP-43 did not affect the dynamics of most RNA granule components such as Pum2 and Stau1 in RNA granules in dendrites, but did affect the dynamics of RNG105 (Caprin1). In contrast to the belief model that FUS and TDP-43 harden RNA granules, the mobility of RNG105 was significantly increased, resulting in the release of RNG105 from RNA granules into the cytoplasm of dendrites. Simultaneously, the amount of mRNAs contained in RNA granules decreased, and local translation in/near the granules also decreased (Figure 2). Since RNG105 is responsible for mRNA recruitment to RNA granules and mRNA transport to dendrites, increased mobility and loss of localization of RNG105 in dendritic RNA granules may affect these functions, leading to impaired synapse formation and cognitive function.

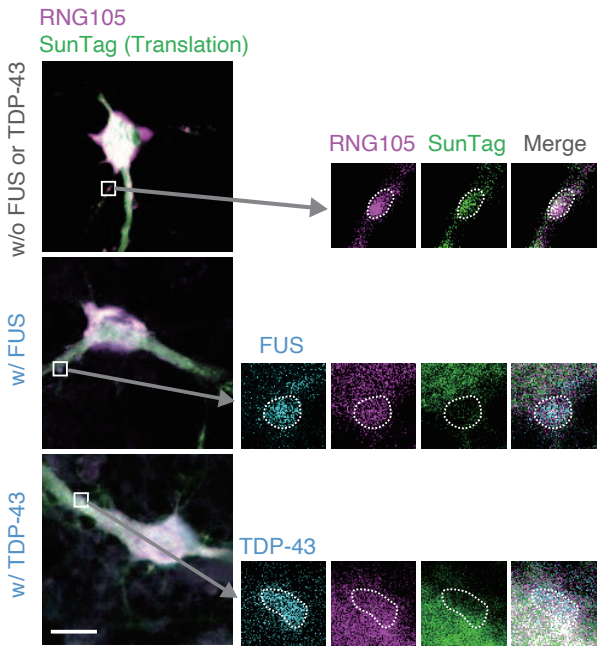


Figure 2. Accumulation of FUS and TDP-43 reduces translation in/near RNG105-localizing RNA granules in dendrites. Primary cultured neurons expressing RNG105-mRFP1 with and without co-expression of FUS-Sirius or TDP-43-Sirius were analyzed using the SunTag system to detect translation activity spatiotemporally. The boxed areas containing dendritic RNA granules are magnified in the right panels. The dotted lines outline the RNA granules. Scale bar, 10 μm.

III. Transport and local translation of mRNAs encoding the regulators of the small G protein Arf in neuronal dendrites

We have previously reported that RNG105 knockout in mice reduces mRNA transport to dendrites and impairs synaptic long-term potentiation and long-term memory (Nakayama et al., *eLife*, 2017). Gene ontology enrichment analysis of mRNA with reduced transport to dendrites in RNG105 knockout mice revealed that a group of mRNAs encoding regulators of the small G protein Arf (Arf GAPs and GEFs) was remarkably reduced in amount in the dendrites of RNG105 knockout neurons. Of these mRNAs, *Psd* mRNA, which encodes an Arf GEF, was most significantly reduced. In order to clarify the biological significance of dendritic transport of *Psd* mRNA, we are aiming to generate mice in which *Psd* mRNA transport can be artificially controlled.

In this study, we identified the 3'UTR of *Psd* mRNA as a cis-element responsible for the transport to dendrites. When *Psd* mRNA lacking the 3'UTR was expressed in mouse primary cultured neurons, the transport of that mRNA to dendrites was reduced. However, it was revealed that the 3'UTR was required not only for dendritic transport but also for translation. Therefore, we narrowed down the responsible regions for the transport and translation in the 3'UTR, and found that 5' half and 3' half of the 3'UTR were required for transport and translation, respectively. Based on these results, we are proceeding with the generation and analysis of mice in which only the 5' half of the 3'UTR of *Psd* mRNA is deleted to reduce only mRNA transport while maintaining the translation level of *Psd* mRNA.

Publication List:

〔Original papers〕

● Nakayama, T., Okimura, K., Shen, J., Guh, Y.-J., Tamai, T.K., Shimada, A., Minou, S., Okushi, Y., Shimmura, T., Furukawa, Y., Kadofusa, N., Sato, A., Nishimura, T., Tanaka, M., Nakayama, K., Shiina, N., Yamamoto, N., Loudon, A.S., Nishiwaki-Ohkawa, T., Shinomiya, A., Nabeshima, T., Nakane, Y., and Yoshimura, T. (2020). Seasonal changes in NRF2 antioxidant pathway regulates winter depression-like behavior. Proc. Natl. Acad. Sci. U.S.A. *117*, 9594–9603. DOI: 10.1073/pnas.2000278117

● Nakazawa, K., Shichino, Y., Iwasaki, S., and Shiina, N. (2020). Implications of RNG140 (caprin2)-mediated translational regulation in eye lens differentiation. J. Biol. Chem. *295*, 15029–15044. DOI: 10.1074/jbc.RA120.012715

〔Review article〕

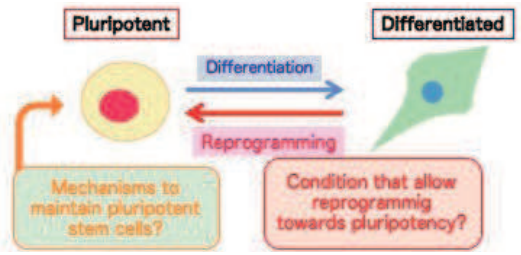
● Ohashi, R., and Shiina, N. (2020). Cataloguing and selection of mRNAs localized to dendrites in neurons and regulated by RNA-binding proteins in RNA granules. Biomolecules *10*. DOI: 10.3390/biom10020167

LABORATORY OF STEM CELL BIOLOGY

Associate Professor
TSUBOUCHI, Tomomi

Specially Appointed Assistant Professor: *KURASHIMA, Kiminori*
SOKENDAI Graduate Student: *KUMAZAKI, Taisei*
MATSUMOTO, Akino
Technical Assistant: *ASAI, Yuriko*
NAGANUMA, Mai

Pluripotent stem cells (PSCs) are defined as stem cells capable of producing all cell types that compose our body. *In vivo,* PSCs appear only transiently during the early stage of development and are lost as development proceeds. Molecular features that underlie pluripotency have been extensively studied, and accumulating evidences suggest that the unique cell cycle regulation of PSC is tightly linked to the maintenance of pluripotency. Curiously, they proliferate with truncated gap phases while lengths of the S (DNA replication) and M (mitosis) phases remain similar to non-pluripotent cell types. In particular, the G1 phase of PSCs needs to be kept short to maintain pluripotency: artificial elongation of the G1 phase makes them susceptible to differentiation. On the other hand, active proliferation with truncated gap phases can cause cellular stress as in some cancer cells, leading to genome instability. Our laboratory is interested in the role of cell cycle regulations in the maintenance of pluripotency and genome integrity of PSCs.



Visual overview of this lab's work.

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"). Recent reports suggest that, compared to other cell types, DNA replication machinery of ES cells proceed at lower speed, a feature generally infer presence of replication obstacles. However, the direct cause and the underlying mechanism remains to be uncovered. 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 cells.

II. Induction of Pluripotency through Nuclear Reprogramming and Role of Cell Cycle Regulation

In mammals, PSCs are not maintained in a fully-developed organism. However, differentiated cells can regain pluripotency upon experimental trigger, albeit at a low efficiency. Factors that limit active reprogramming, and conditions that potentiate reprogramming, are the subjects under active investigation.

One of the main obstacles when investigating molecular mechanism underlying reprogramming is the time it takes to start seeing any sign of reprogramming. In order to overcome this problem, 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, in a short time frame. 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 within one day after fusion, thus allowing us to monitor the initial events of reprogramming after induction.

Using this system, we previously found that DNA synthesis is an important event for successful reprogramming (Tsubouchi *et al*., Cell, 2013). Recent reports indicate that reprogramming may cause genetic instability, some of which are thought to arise as DNA replication errors. To investigate the nature of such errors and how they are linked to repro-

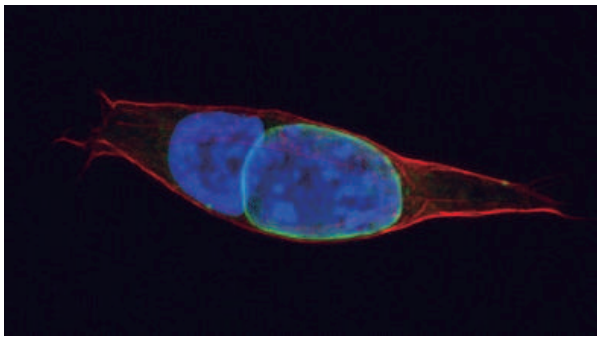


Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus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

gramming-specific events, we have set up a system to isolate and track a single fused cell (Figure 1) through live-imaging.

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 its identity is being altered (through differentiation, reprogramming etc.) are largely 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 gain a comprehensive understanding of how genome integrity is maintained in ES cells and cells undergoing reprogramming.

Publication List:

〔Review article〕

- Tsubouchi, T., and Pereira, C.-F. (2021). Reprogramming Stars #1: Genome Programming Through the Cell Cycle-An Interview with Dr. Tomomi Tsubouchi. Cell. Reprogram. *23*, 153–157. DOI: 10.1089/cell.2021.29039.tt

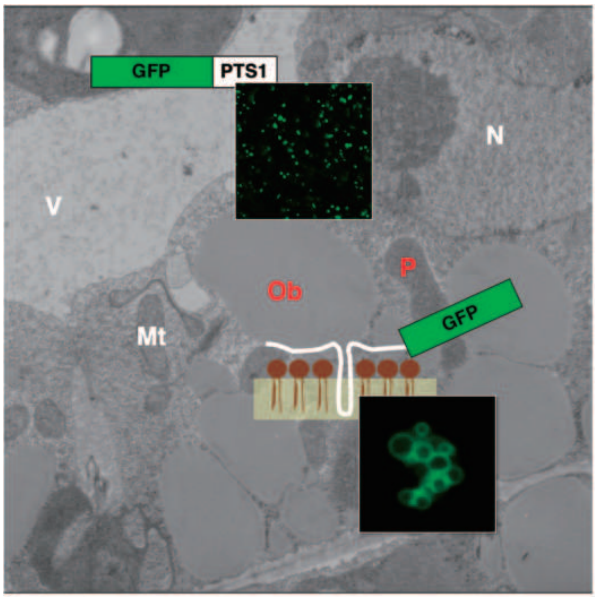
LABORATORY OF ORGANELLE REGULATION

Associate Professor
MANO, Shoji

Specially Appointed Assistant Professor: *KANAI, Masatake*
Visiting Scientist: *KAMIGAKI, Akane*
OIKAWA, Kazusato
Technical Assistant: *HIKINO, Kazumi*
HOSHI, Rie
Admin Support Staff: *UEDA, Chizuru*
ASAI, Sanae

Because plants spread their roots in the ground, they must survive in a given environment. To adapt to their environment, they utilized various signals generated from environmental changes as being necessary for their survival. As such, the flexibility of plant organelles is the basis for such adaptation.

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.



Visual overview of this lab's work.

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

Peroxisomes are single-membrane-bound organelles, which are frequently present in eukaryotic cells, and are involved in various biological processes such as lipid metabolism and photorespiration. These functions change dramatically during certain developmental stages, and when they are 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 during 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 functions.

To better understand peroxisome biogenesis and functions, we isolated a number of Arabidopsis mutants that displayed aberrant peroxisome morphology (*apem* mutants) and unusual peroxisome 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 (Figure 1). As of writing, we have reported the functions 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*, *peup4*, *peup17* and *peup22* mutants, which were defective in *Autophagy-related* (*ATG*) genes.

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

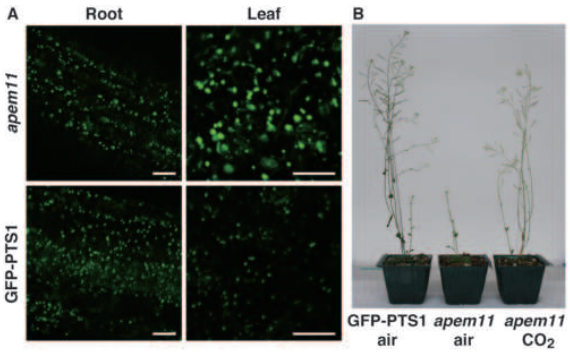


Figure 1. Phenotype of Arabidopsis *apem11* mutants. (A) GFP fluorescence was observed in the parent plant, GFP-PTS1, and *apem11* mutants. The *apem11* mutant has enlarged peroxisomes in leaf cells, but not in root cells. Bars, 20 μm. (B) The *apem11* mutant showed dwarfism under normal atmosphere, but grew normally under high CO2 atmosphere as did the GFP-PTS1 plant.

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 proteins are synthesized in the endoplasmic reticulum (ER) and accumulated in oil and protein bodies, respectively, during seed development.

We are currently analyzing the molecular mechanisms controlling oil and protein contents in seeds (Figure 2). 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 transcription factor in fatty acid biosynthesis, expression during the mid-phase of seed development significantly enhanced seed oil content, and caused seed sizes to enlarge.

We are also 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 a mechanism required for the quality control of fatty acids by degrading particular fatty acids in oil bodies.

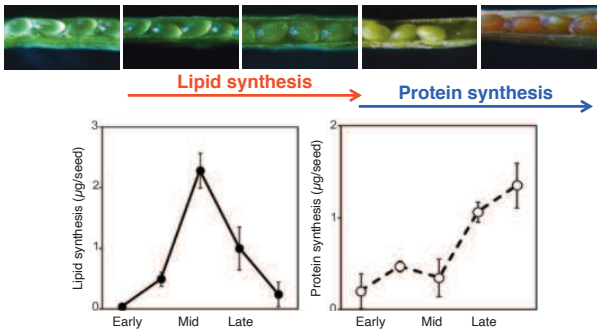


Figure 2. Biosynthesis of lipids and proteins during Arabidopsis seed development. Lipid synthesis and accumulation begins before those of seed storage proteins during seed development.

III. Development of Gateway-technology vectors for plant research

Gateway cloning is a useful and powerful 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. Up until now, 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 (Figure 3), and, as a public database, it 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 concerning 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 structural rotations
- The organellome database is a compilation of static image data of various tissues of several plant species at different stages of development.
- The functional analysis database is a collection of protocols for plant organelle research

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

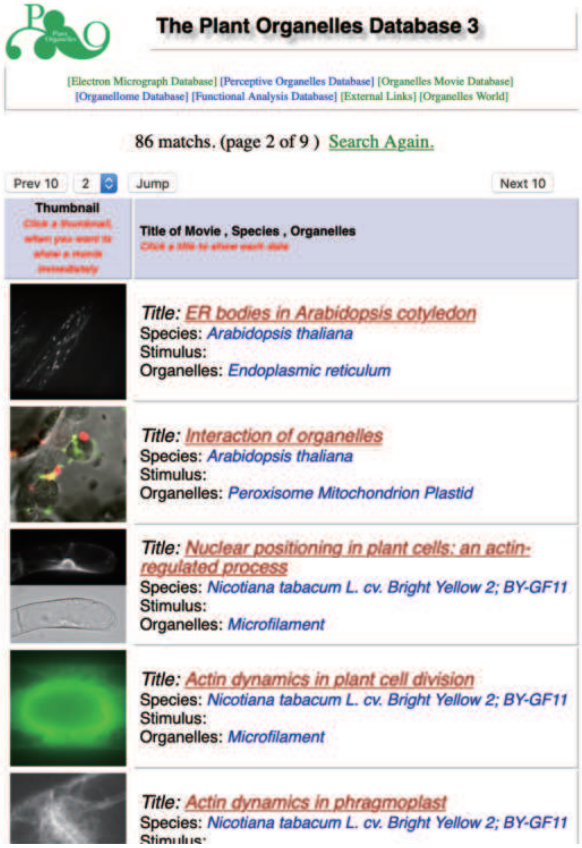


Figure 3. The graphical user interface of the PODB3 (http://podb.nibb.ac.jp/Organellome).

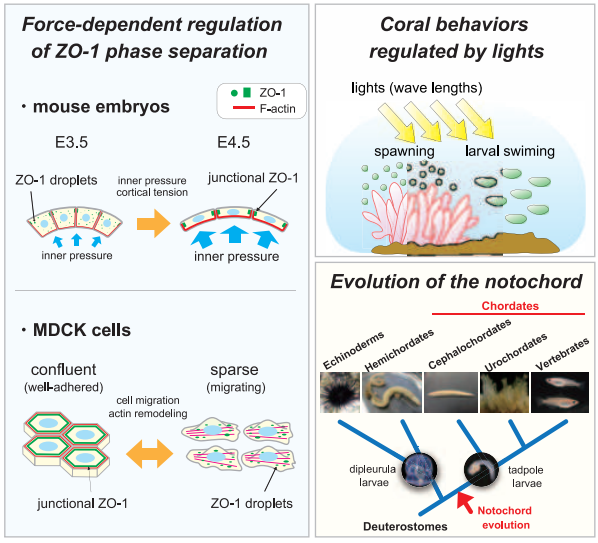
DIVISION OF MORPHOGENESIS

Professor
UENO, Naoto

Associate Professor
KINOSHITA, Noriyuki

Assistant Professor: *TAKAHASHI, Hiroki*
Technical Staff: *TAKAGI, Chiyo*
Technical Assistant: *YAMAMOTO, Takamasa*
YASUE, Naoko
Admin Support Staff: *MIYAKE, Satoko*
TSUGE, Toyoko

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 both cellular and organismal behaviors. In particular, we are currently investigating the contribution of physical forces using mouse embryos and light-dependent behaviors using coral larvae to understand the impact of physical environments on organisms. As reported below, the inner pressure of the blastocyst cavity generates tensile force upon trophectodermal cells in mouse embryos, and enhances their cell junction. During this process, a physical phenomenon, liquid-liquid phase separation (LLPS) of the tight junction protein ZO-1, plays a pivotal role. The coral planula larvae change their swimming activities by sensing types of light of specific wavelengths to settle upon an appropriate place in the sea in which to live. We also investigate the evolution of the notochord using ancestral chordates.



A graphical overview of our laboratory's works.

I. ZO-1 condensates in mouse hatching embryos

Cells sense and respond to not only molecular factors but also mechanical forces, which in turn play various important roles in biological events, such as tissue homeostasis, differentiation, and cell migration. During early embryogenesis, various dynamic morphogenetic movements occur, including the convergent extension of the axial mesoderm and epiboly of the ectoderm. These movements generate physical forces at both the cellular and tissue levels. However, it is still not fully understood how these forces influence morphogenetic processes.

To investigate how embryonic cells respond to mechanical stimuli, we artificially applied mechanical forces to *Xenopus* embryos. We found that tensile force applied to ectodermal tissue induces a mesenchymal-epithelial transition (MET)-like phenotype, enhancing the junctional structure and increasing ectodermal tissue stiffness. Among these changes, we focused on the behavior of the tight junction protein, ZO-1. Before the force application, ZO-1 forms cytoplasmic granules, but the mechanical force induces its accumulation at the tight junction. Significantly, mechanical forces generated by gastrulation movements also induce similar changes in the developing ectodermal tissue.

To examine whether the behavior of ZO-1 protein is conserved across species, in mammals in particular, we first observed the localization of ZO-1 in the mouse embryo. We focused on E3.5 and E4.5 embryos since they hatch out of the zona pellucida (ZP) and expand their shape (Figure 1A). We immunostained E3.5 and E4.5 embryos with a ZO-1 antibody and found that the cells of E3.5 embryos showed a significantly higher number of ZO-1 puncta in the cytoplasm relative to E4.5 embryos. As development proceeded, the surface area of the trophectoderm (TE) cells expanded and became thinner. The number of cytoplasmic ZO-1 puncta was reduced, and ZO-1 signal intensity at the

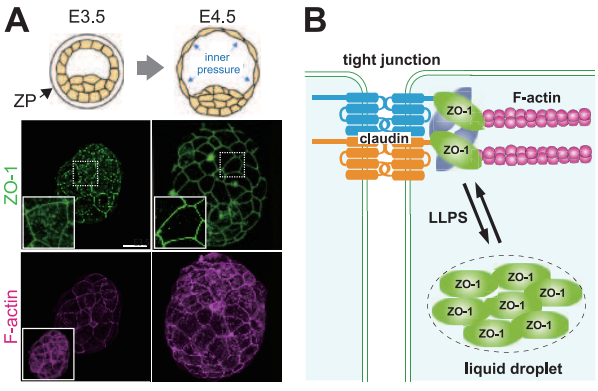


Figure 1. Change of ZO-1 localization in mouse hatching embryos.
A. At E3.5, the embryo is covered with the zona pellucida (ZP). During hatching, the embryo is enlarged and emerges from the ZP. Embryos were stained with an anti-ZO-1 antibody and Alexa Fluor 546 Phalloidin. Scale bar, 20 μm. The inset in the E3.5 phalloidin image was acquired with higher laser power, demonstrating that the structure of cortical F-actin is formed at E3.5 even though the signal intensity was weaker than that in the E4.5 embryo. B. Schematic diagram of ZO-1 behavior shuttling between the tight junction and cytoplasmic droplets.

plasma membrane in E4.5 embryos became much higher than those of E3.5 embryos at the expense of their cytoplasmic pool. Importantly, this change coincides well with the accumulation of F-actin in the cell cortex in E4.5 embryos. This result suggests that the shuttling of ZO-1 protein from the cytoplasmic puncta to cell junctions occurred as development progressed.

To confirm that the tensile force induces this ZO-1 behavior in TE cells, we inhibited the expansion of said cells using two methods: one was the Na^+/K^+ ATPase inhibitor, ouabain treatment, and the other was piercing the embryo with a glass needle. Both methods reduced the inner pressure of the cavity and inhibited ZO-1 accumulation in the cell junction. These results suggested that tensile force being applied to the TE cells. Furthermore, we assumed that the ZO-1 puncta in E3.5 embryos were formed by phase separation and treated embryos with 1,6-hexanediol, which is known to dissolve LLPS assembly. As expected, 5% 1,6-hexanediol treatment reduced the number of particles within a few minutes, thus suggesting that these puncta are liquid droplets generated by phase separation, and that mechanical force regulates ZO-1 phase separation (Figure 1B).

II. Interaction with F-actin regulates ZO-1 phase separation

To analyze the nature of ZO-1 liquid droplets in various cell environments, we first expressed GFP-ZO1 in A6 cells. GFP-ZO-1 is mainly localized in the cell periphery and colocalized with F-actin bundles. We thus treated cells with latrunculin B, which disrupted F-actin (Figure 2B). We found that the formation and extinction of cytoplasmic ZO-1 granules depended on the destruction and development of the F-actin network. Furthermore, we confirmed that latrunculin B-induced ZO-1 granules rapidly recovered in the FRAP assay. These observations indicate that the efficiency and growth of ZO-1 condensation are negatively regulated by the interaction with F-actin in A6 cells.

To inhibit the interaction between ZO-1 and F-actin in a different way, we constructed GFP-ZO-1ΔABD, which lacks the actin-binding domain (ABD) (Figure 2A), and expressed it in A6 cells. GFP-ZO-1ΔABD subsequently formed droplets with a smooth surface (Figure 2C). This suggests that cytoplasmic droplet formation is restricted by the binding of ZO-1 to F-actin and that GFP-ZO-1ΔABD lost its capacity for the interaction. It is known that when proteins form liquid droplets, intrinsically disordered regions (IDRs) play an important role in phase separation. In human ZO-1, four IDRs are predicted (Figure 2A). Among these, we found that the C-terminal IDR4, is essential for phase separation. Since IDR4 includes ABD, binding to F-actin may affect the LLPS-inducing activity of the IDR4.

We observed ZO-1 behavior in the wound healing assay using canine MDCK cells. Initially, most of the cells had few condensates in a confluent cell sheet. However, after scratching, cells started active migration toward the wound site, forming a significant number of droplets (Figure 2D). In these cells, F-actin distribution and intensity also changed (Figure 2E). Initially, cortical F-actin was well-developed in the non-migrating polygonal cells with junctional ZO-1. In contrast, migrating cells around the wound site, ones that were forming ZO-1 droplets, reduced cortical actin and developed more stress fibers. This correlation between ZO-1 and F-actin is consistent with our previous finding that the mouse E3.5 embryo with ZO-1 droplets had less F-actin than the E4.5 embryo with junctional ZO-1. Thus, ZO-1's phase separation is regulated by cell-cell interaction and F-actin remodeling.

We also analyzed LLPS using bacterially-produced ZO-1 *in vitro*. We found that ZO-1 IDR4 was sufficient to form liquid droplets *in vitro* (Figure 2F), and the full-length ZO-1 can bind to F-actin (Figure 2G). This system will be useful in analyzing detailed molecular mechanisms of ZO-1 phase separation.

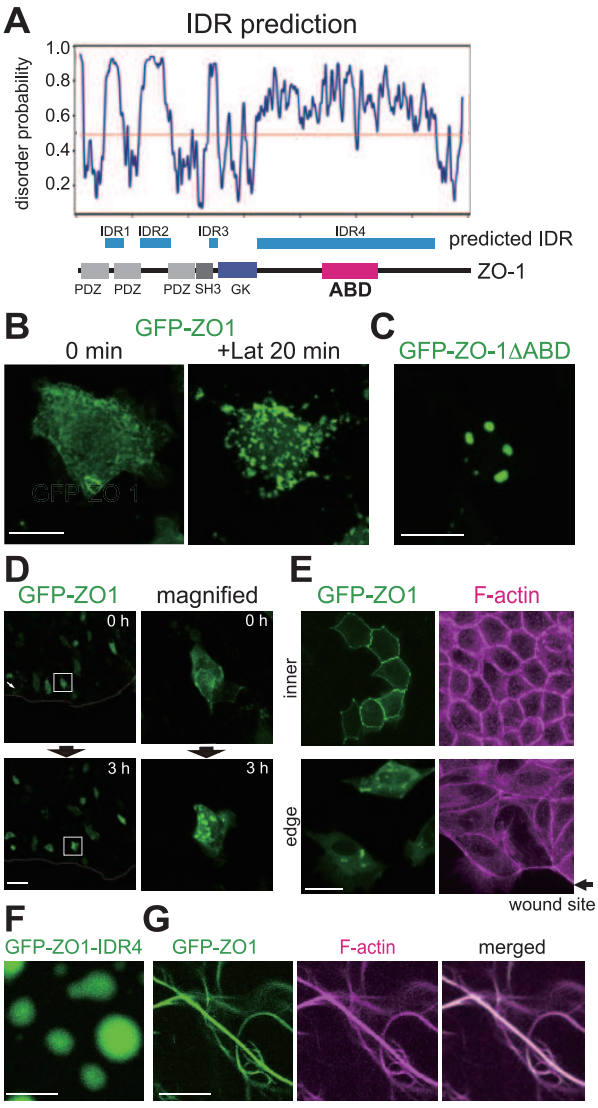


Figure 2. Interaction with F-actin regulates ZO-1 phase separation.
A. The domain structure and the intrinsically disordered regions (IDRs) of ZO-1. B. A6 cells were treated with latrunculin B (Lat) to disrupt F-actin. Bar = 20 μm. C. GFP-ZO-1 lacking the actin binding domain (ABD) was expressed in A6 cells. Bar = 20 μm. D. A wound healing assay using MDCK cells. Bar = 50 μm. E. 10 hours after the wound healing assay, cells close to the wound site ("edge") and in the inner region ("inner") were stained with phalloidin. Bar = 20 μm. F. Purified GFP-ZO1-IDR4 formed liquid droplets in vitro. Bar = 5 μm. G. Purified GFP-ZO-1 interacts with F-actin. Bar = 10 μm.

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

The notochord and somite are the most central organs in chordates, and Brachyury (Bra) plays a pivotal role in their formation. The question of how Bra gained its enhancer activity in the notochord-specific expression is critical to our understanding of chordate evolution. In cephalochordates, 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 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 occurred in the notochord, whereas both muscle and the notochord indicated BfBra2 expression. 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 was 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.

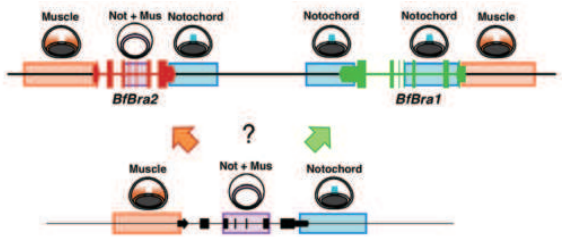


Figure 3. A summary of results of the present reporter assay and their possible interpretation.
Enhancer activity of 5' upstream sequences in muscle is shown in brown and that of the 3' downstream in notochord is in blue. The activity of *BfBra2* introns in muscle and notochord is shown in purple.

IV. A step-down photophobic response in larvae of the common reef coral, *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 the light in their environment remains largely unknown. To answer these questions, we analyzed the swimming behavior of larvae of the common reef coral Acropora tenuis (Figure 4) under various light conditions. In addition, we developed a mathematical model to test whether the observed light response resulted in aggregation or dispersal under specific light fields.

First, we precisely observed the larval swimming activity under fluctuating light conditions and found that larvae temporarily stopped swimming ~30 s after rapid light intensity reduction, thus exhibiting a step-down photophobic response. This behavior was also observed when we rapidly changed the spectral composition of light. Further experiments using the Okazaki Large Spectrograph revealed that the loss of short wavelengths of light (blue/green light) induced this type of behavior.

The analysis of mathematical simulations of this step-down photophobic response indicates that larvae will aggregate in the lighter areas of two-dimensional large rectangular fields. These results suggest that the step-down photophobic response of coral larvae may play an important role in determining where larvae settle on the reef.

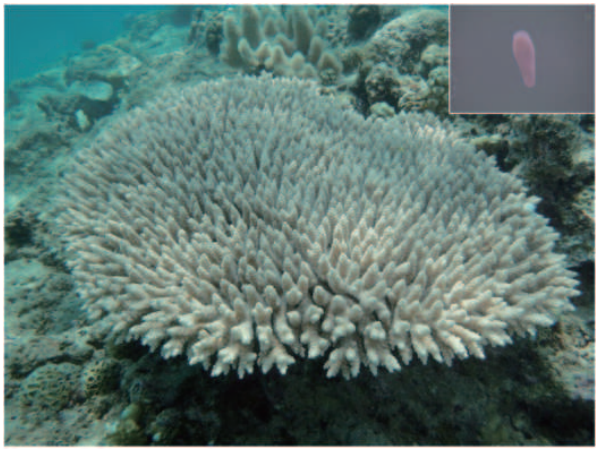


Figure 4. A wild colony of *Acropora tenuis* (photo taken by Masayuki Hatta at Ochanomizu University). The inset shows a planula larvae of *A. tenuis*.

Publication List:

〔Original papers〕

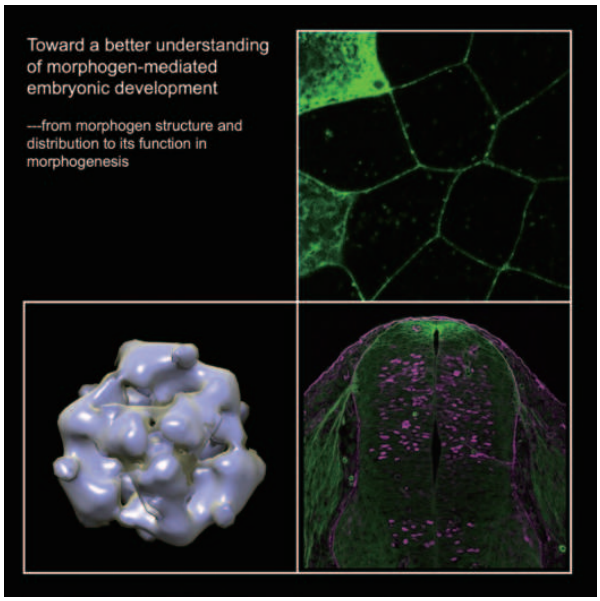
- Kinoshita, N., Yamamoto, T.S., Yasue, N., Takagi, C., Fujimori, T., and Ueno, N. (2022). Force-dependent remodeling of cytoplasmic ZO-1 condensates contributes to cell-cell adhesion through enhancing tight junctions. iSCIENCE *25*, 103846. DOI: 10.1016/j.isci.2022.103846
- Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y., and Aoki, K. (2021). Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. Nat. Commun. *12*, 7145. DOI: 10.1038/s41467-021-27458-3

DIVISION OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

Professor
TAKADA, Shinji

Assistant Professor:	*YABE, Taijiro*
	MII, Yusuke
Specially Appointed Assistant Professor:	
	SHINOZUKA, Takuma
Technical Staff:	*UTSUMI, Hideko*
Postdoctoral Fellow:	*TAKADA, Ritsuko*
SOKENDAI Graduate Student:	*HATAKEYMA, Yudai*
	TRAN, Thi Hong Nguyen
	SUZUKI, Minako
Technical Assistant:	*TAKASHIRO, Kayoko*
	ITO, Yukiko
	SAKAI, Kimiko
Admin Support Staff:	*NOBATA, Ryuko*

The morphology of the body and tissues is established in a spatio-temporarily regulated manner. A number of genes involved in the process of 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 and periodical information is converted into morphology through the application of several different approaches.



Visual overview of this lab's work.

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 is yet to be elucidated. To this end, we have visualized signal proteins and monitored their movement in tissues. Furthermore, we are also 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 research 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.

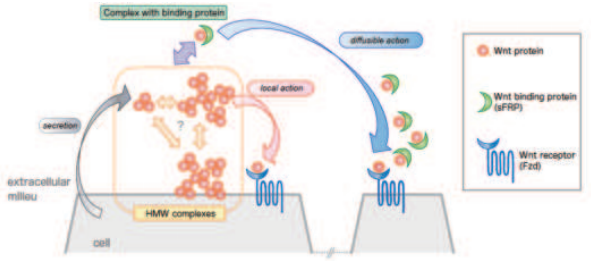


Figure 1. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion 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 proteins (partner proteins), including sFRP. Due to this dissociation, Wnt turns out to be more mobile and its diffusion range is expanded (diffusible action).

I. Regulation of spatial distribution and function of Wnt proteins in vertebrates

By combining biochemical and structural analyses, we have already shown that Wnt3a proteins are not secreted in a monomeric form, rather in homo-trimer and larger HMW complexes. Secreted Wnt3a proteins were able to be dissociated via interaction with their receptor Frizzled8 and with a secreted Wnt binding protein, sFRP2, *in vitro*. Similarly, this dissociation was detected *in vivo* by Fluorescence

Correlation Spectroscopy (FCS). Several lines of evidence show that large assemblies of Wnt3a are less mobile, and Wnt/sFRP2 heterodimer, which is generated through the binding of dissociated Wnt with sFRP2, diffuse more freely. Based on these results, we have proposed a model which contends that the assembly and dissociation of dissociable oligomers modulate Wnt signaling range (Figure 1).

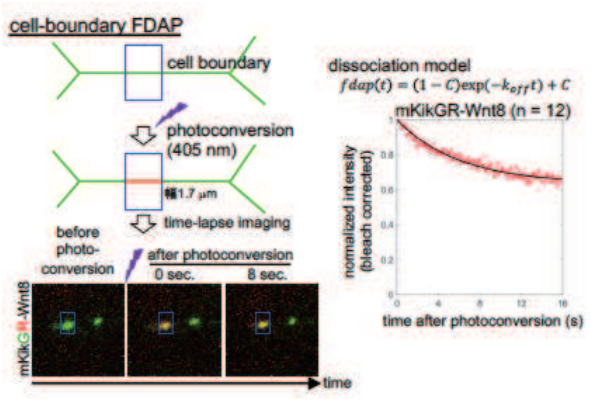


Figure 2. Fluorescence decay after photoconversion (FDAP) at cell coundaries. A Photoconvertable fluorescent protein, mKikGR can be switch its fluorescence from green to red. Decay of red (photoconverted) fluorescence can be fitted with a model of dissociation from cell surface scaffolds.

To increase our insight into the intercellular transmission of Wnt proteins in embryonic tissue, we precisely examined the extracellular dynamics of Wnt, comparing with sFRP in Xenopus embryos. Here, we focused on Wnt8 and a member of sFRPs, Frzb, both of which are involved in the anteroposterior patterning of the vertebrate embryo. While Venus-tagged Wnt8 was found on the surfaces of cells close to Wnt-producing cells, we also detected its dispersal over a long range from the source cells. We further examined their dynamics by FCS and fluorescence decay after photoconversion (FDAP)-based measurements in the embryonic tissue. In particular , we refined FDAP-based analysis by focusing on a limited area across the cell surface, which enabled us to obtain dynamics comparable to those measured by FCS (Figure 2). Combination of fluorescence correlation spectroscopy and quantitative imaging revealed that only a small proportion of Wnt8 proteins diffuse freely, whereas most of them are bound to the cell surface. FDAP analysis, that we refined by focusing on a limited area across the cell surface, showed that Wnt8 proteins that were bound to the cell surface were rapidly and exponentially decreasing, suggesting a dynamic exchange of a bound form of Wnt proteins. Based on these results and our previous findings, we have proposed a basic mathematical model to explain distribution and dispersion of secreted proteins (Figure 3). This model, which is based on the dynamic exchange of the bound form of Wnt proteins, can recapitulate a graded distribution of the bound, not free, state of Wnt proteins.

In an attempt to investigate the physiological role of Wnt signaling, we collaborated with Dr. Fujimori and Dr. Takahama at the University of Tokushima and NIH, respectively, to examine gain-of-function (GOF) and loss-of-function (LOF) of β-catenin highly specific in mouse thymic epithelial cells (TECs). GOF of β-catenin in TECs results in severe thymic dysplasia and T-cell deficiency beginning from the embryonic period. By contrast, loss-of-function of β-catenin in TECs reduces the number of cortical TECs and thymocytes modestly and only postnatally. These results indicate that fine-tuning of β-catenin expression within a permissive range is required for TECs to generate an optimal microenvironment to support postnatal T-cell development.

II. The molecular mechanism of metameric structures in vertebrate development

The segmental sub-regions of several specific tissues, including the paraxial mesoderm (or somites) and the pharyngeal pouches is not likely to be simply directed by the gradient of the signal molecules, but by a unique mechanism proceeding periodically. For instance, somites are sequentially generated in an anterior-to-posterior order by converting the temporal periodicity created by a molecular clock into periodical structures. The molecular mechanism underlying this conversion and morphological segmentation, however, is not yet fully understood.

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. The molecular clock, the so-called segmentation clock, essentially creates oscillatory expression of particular genes, *hairy* and some *notch*-related genes, in the posterior PSM.

As the oscillatory waves of the segmentation clock move from posterior to anterior PSM, the temporal dynamics of the segmentation clock are transformed into the spatial pattern

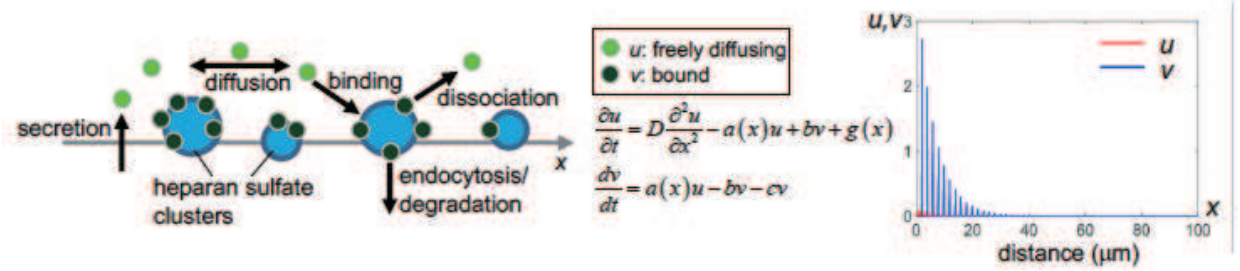


Figure 3. Mathematical model considering free diffusion and binding to heparan sulfate clusters. The free population (*u*) does not virtually contribute to spatial distribution, whereas the bound population (*v*) directly contributes to the graded distribution of a morphogen. This model suggests both populations contribute to rapid formation of a stable gradient, which gives insight into a perceived dilemma between speed and stability of gradient formation.

of somites. One of the key processes shaping the spatial pattern of somites is the generation of inter-somite boundaries. Evidences indicate that inter-somite boundary is defined by the anterior edge of the Tbx6 protein expression region. It has been reported by us and other groups that this anterior border is newly generated in each segmentation cycle, by periodic degradation of Tbx6 protein via physical interaction with *Ripply1* and *Ripply2*.

We are currently using a genetic approach in zebrafish to elucidate the mechanisms of the interaction between the segmental clock and the Tbx6/Ripply system. Based on the results obtained from the genetic approach, we provide a mathematical model showing the minimal network that forms the metameric pattern of somites and the periodic cessation of the segmentation clock.

Publication List:

〔**Original papers**〕

- Feng, D., Wang, J., Yang, W., Li, J., Lin, X., Zha, F., Wang, X., Ma, L., Choi, N.T., Mii, Y., Takada, S., Huen, M.S.Y., Guo, Y., Zhang, L., and Gao, B. (2021). Regulation of Wnt/PCP signaling through p97/VCP-KBTBD7-mediated Vangl ubiquitination and endoplasmic reticulum-associated degradation. Sci. Adv. *7*, eabg2099. DOI: 10.1126/sciadv.abg2099
- Fujimori, S., Ohigashi, I., Abe, H., Matsushita, Y., Katagiri, T., Taketo, M.M., Takahama, Y., and Takada, S. (2022). Fine-tuning of beta-catenin in mouse thymic epithelial cells is required for postnatal T-cell development. eLife *11*, e69088. DOI: 10.7554/eLife.69088
- Matsuda, S., Schaefer V, J., Mii, Y., Hori, Y., Bieli, D., Taira, M., Plueckthun, A., and Affolter, M. (2021). Asymmetric requirement of Dpp/BMP morphogen dispersal in the Drosophila wing disc. Nat. Commun. *12*, 6435. DOI: 10.1038/s41467-021-26726-6
- Mii, Y., Nakazato, K., Pack, C.-G., Ikeda, T., Sako, Y., Mochizuki, A., Taira, M., and Takada, S. (2021). Quantitative analyses reveal extracellular dynamics of Wnt ligands in *Xenopus* embryos. eLife *10*, e55108. DOI: 10.7554/eLife.55108
- Okubo, T., Hara, K., Azuma, S., and Takada, S. (2021). Effect of retinoic acid signaling on Ripply3 expression and pharyngeal arch morphogenesis in mouse embryos. Dev. Dyn. *250*, 1036–1050. DOI: 10.1002/dvdy.301
- Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y., and Aoki, K. (2021). Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. Nat. Commun. *12*, 7145. DOI: 10.1038/s41467-021-27458-3

〔**Review article**〕

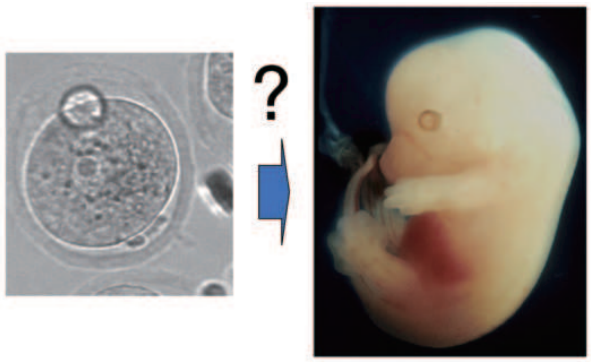
- Shinozuka, T., and Takada, S. (2021). Morphological and functional changes of roof plate cells in spinal cord development. J. Dev. Biol. *9*, 30. DOI: 10.3390/jdb9030030

Division of Embryology

Professor
FUJIMORI, Toshihiko

Associate Professor: *NONOMURA, Keiko*
Assistant Professor: *KOYAMA, Hiroshi*
Specially Appointed Assistant Professor: *ARATA, Masaki*
Technical Staff: *OKA, Sanae*
Postdoctoral Fellow: *KISHI, Kanae*
SOKENDAI Graduate Student: *SAKURAI, Jun*
Visiting Graduate Student: *MIKOSHIBA, Seiya*
Visiting Scientist: *INOUE, Yuuki*
Technical Assistant: *HIGUCHI, Yoko*
KANIE, Akemi
NAKAGAWA, Mami
Admin Support Staff: *KATO, Azusa*

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.



A mouse fertilized egg and an embryo at 12 days after fertilization. Embryonic body with axes of anterior-posterior, dorsal-ventral and left-right is formed from an egg with symmetrical shape. How is the information relating future body formed during early stages of development?

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.

In addition to these reporter mouse lines, we generated transgenic (Tg) mice expressing photoactivatable-Cre (PA-Cre) proteins. Due to the limitation of high throughput analyses in mouse embryos, we established embryonic stem cells from the Tg mice for testing various conditions. We then systematically investigated the conditions which enable efficient photoactivation for PA-Cre, in vitro and in silico. It was suggested that repetitive pulsed illumination with short pulse cycles efficiently induced DNA recombination with low light energy. We also showed that repetitive short-pulsed illumination induced DNA recombination in pre-implantation embryos expressing PA-Cre.

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.

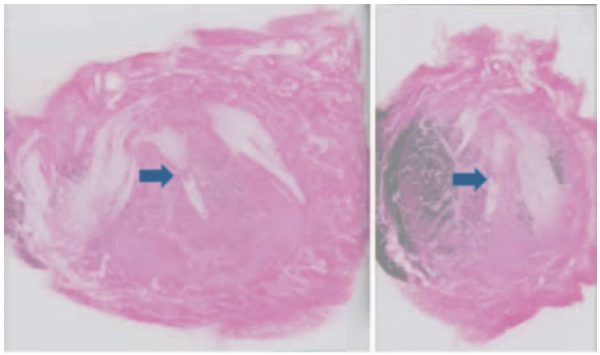


Figure 1. Three-dimensional image reconstruction of the pregnant uterus. A uterus obtained 5 days after fertilization was sectioned and images were digitized by an image scanner, and reconstructed three-dimensionally to observe the relationship between the embryo and the maternal tissue. The right panel 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. 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. Recently, we found that Camsap3, a microtubule minus-end binding protein, localized to the uterine side of the base of cilia and its mutation disrupted cilia orientation in individual cells without affecting Celsr1 localization. Thus, both Celsr1 and Camsap3 are responsible for the alignment of cilia along the ovary-uterus axis, but in distinct ways; Celsr1 orients cells, while Camsap3 orients cilia within each cell.

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.

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

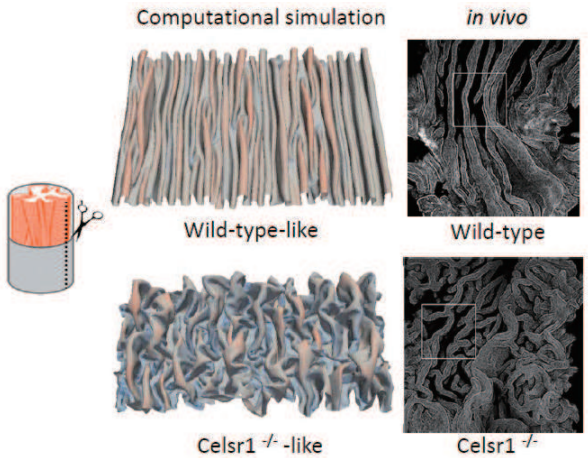


Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

(Figure 3). By employing this method, we inferred mechanical forces in multi-cellular systems including cultured epithelial cells, and early embryogenesis in *C. elegans* and mice. Further computational simulations based on the inferred mechanical information reproduced morphological features of the multi-cellular systems. Thus, the mechanical information will be useful to investigate physical mechanisms of early embryonic development and morphogenesis during organogenesis in late stages of development.

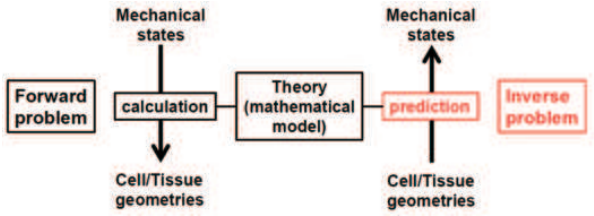


Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

V. Mammalian tissue morphogenesis requiring mechanosensor channel PIEZOs

Several examples have shown that mechanical stimuli can work as key components for tissue or organ development. However, our knowledge about the involvement of mechanotransduction in biological phenomena or their precise mechanisms is still limited. It is partially because key mechanosensors are not yet identified in many cell types. PIEZOs are recently identified mechanically activated cation channels functioning in mammalian cells (Figure 4). They are activated when mechanical forces are applied to the cell membrane. Series of data show that PIEZO2 serves as the main mechanosensor in sensory neurons for light touch 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.

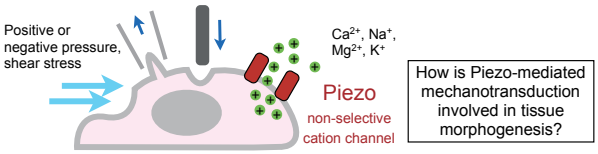


Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

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.

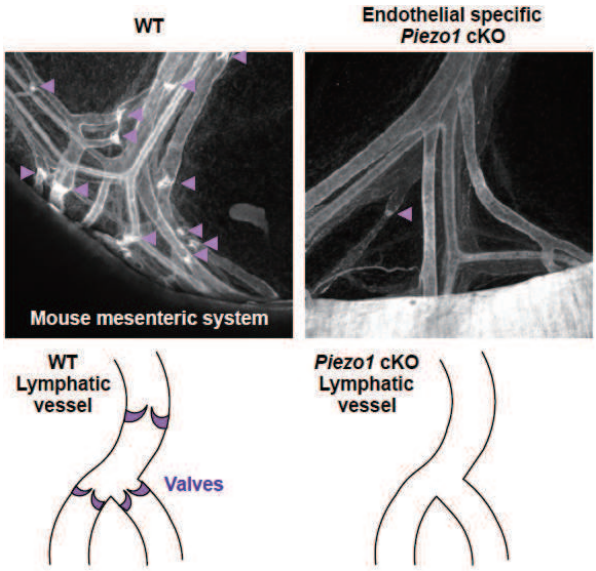


Figure 5. Reduced number of lymphatic valves in mice lacking PIEZO1 in endothelial cells.

Publication List:

〔Original papers〕

- Hirata, T., Tohsato, Y., Itoga, H., Shioi, G., Kiyonari, H., Oka, S., Fujimori, T., and Onami, S. (2021). NeuroGT: A brain atlas of neurogenic tagging CreER drivers for birthdate-based classification and manipulation of mouse neurons. Cell Reports Methods *1*, 100012. DOI: 10.1016/j.crmeth.2021.100012
- Kinoshita, N., Yamamoto, T.S., Yasue, N., Takagi, C., Fujimori, T., and Ueno, N. (2022). Article Force-dependent remodeling of cytoplasmic ZO-1 condensates contributes to cell-cell adhesion through enhancing tight junctions. iScience *25*, 103846. DOI: 10.1016/j.isci.2022.103846
- Kishi, K., Koyama, H., Oka, S., Kato, A., Sato, M., and Fujimori, T. (2021). Repetitive short-pulsed illumination efficiently activates photoactivatable-Cre as continuous illumination in embryonic stem cells and pre-implantation embryos of transgenic mouse. GENESIS *59*, e23457. DOI: 10.1002/dvg.23457
- Koyama, H., Suzuki, M., Yasue, N., Sasaki, H., Ueno, N., and Fujimori, T. (2022). Differential Cellular Stiffness Contributes to Tissue Elongation on an Expanding Surface. Front. Cell Dev. Biol. *10*, 864135. DOI: 10.3389/fcell.2022.864135
- Saito, H., Matsukawa-Usami, F., Fujimori, T., Kimura, T., Ide, T., Yamamoto, T., Shibata, T., Onoue, K., Okayama, S., Yonemura, S., Misaki, K., Soba, Y., Kakui, Y., Sato, M., Toya, M., and Takeichi, M. (2021). Tracheal motile cilia in mice require CAMSAP3 for the formation of central microtubule pair and coordinated beating. Mol. Biol. Cell *32*. ar12. DOI: 10.1091/mbc.E21-06-0303

DIVISION OF GERM CELL BIOLOGY

Professor
YOSHIDA, Shosei

Assistant Professor:	*KITADATE, Yu*
	NAKAGAWA, Toshinori
Specially Appointed Assistant Professor:	
	SUZUKI, Shinnosuke
Technical Staff:	*MIZUGUCHI, Hiroko*
JSPS Postdoctoral Fellow:	*IKEDA, Tatsuro*
Postdoctoral Fellow:	*HIRANO, Kodai*
	SATO, Toshiyuki
Visiting Graduate Student:	*BABAGUCHI, Hirotaka*
Technical Assistant:	*FUJITA, Miyako*
	NISHIMURA, Chiaki
Admin Support Staff:	*KUBOKI, Yuko*

Our research

Many animals, including mammalians, produce a large number of sperm over a long period for reliable reproduction. On the other hand, each sperm correctly replicates and transmits the genetic information to the next generation. The ultimate goal of the Division of Germ Cell Biology is to reveal how seemingly contradictory, albeit essentially important, productivity and fidelity are both achieved. To this end, we pay special attention to the sperm stem cells. We are also interested in other aspects of spermatogenesis.

What are the sperm stem cells?

The continual sperm production is supported by the "sperm stem cells," termed formally "SSCs" after spermatogonial or spermatogenic stem cells. SSCs maintain a delicate balance between self-renewal and differentiation to sustain life-long spermatogenesis. It has been a central question which fraction of *spermatogonia*, the "early stage of cells before meiosis, are SSCs and where and how they behave in the testis.

The backbone of mammalian SSC research was established from the 1950s to the 1970s, relying on morphological analyses of fixed testis specimens. This led to a proposal of the prevailing stem cell model called the "A_s model." This model proposed that singly isolated spermatogonia (A-single or A_s cells) act as SSCs. On the other hand, all syncytia are all committed to differentiation.

Our lab has introduced the *scale of time* using updated technologies, *i.e.*, live-imaging and pulse-labeling studies, which revealed many different aspects of dynamical behaviors of SSCs (Figure. 1).

A_s cells and syncytia together comprise a single SSC pool

Our live-imaging studies combined with mathematical and statistical analysis evidenced that SSCs reversibly change their morphology between singly-isolated and syncytia. This is mediated by incomplete cell division and breakdown of syncytia (Nakagawa et al., Science 2010; Hara *et al*., Cell Stem Cell 2014).

Cells inclined for differentiation can revert to SSCs

It is generally thought that once stem cells have been destined for differentiation, they never replicate themselves again. We found that such cells maintain the potential to self-renew up to a particular stage of differentiation and revert to SSCs with high frequency, particularly during regeneration after tissue damage (Nakagawa *et al*., Developmental Cell 2007).

SSCs follow variable fate behaviors

Stem cells are often thought to invariably undergo asymmetric division, which produces one stem cell and one differentiating cell to maintain homeostasis. However, we found that individual SSCs follow highly variable fates. Still, collectively, they achieve a perfect balance between self-renewal and differentiation at a population level (Klein *et al*., Cell Stem Cell 2010; Hara *et al*., Cell Stem Cell 2014).

SSCs show active motion in an "open niche"

In many tissues, stem cells receive self-renewing signals in defined niche regions. We found that the mouse testis has no such areas, with SSCs scattered among differentiated cells and moving around actively. Further, their distribution is biased near blood vessels. Such microenvironment is called an "open (or facultative) niche" in contrast to the afore-mentioned "closed (or defined) niche" (Yoshida *et al*., Science 2007; Hara *et al*., Cell Stem Cell 2014).

SSC heterogeneity underpins the asymmetric fates of SSCs

In an open niche, some SSCs remain undifferentiated while others differentiate in response to broadly distributed signaling molecules. We found that some SSC fractions resist differentiation-promoting Wnt signal by expressing a Wnt-inhibitor, Shias 6. Another subset responds to retinoic acid (RA) by upregulating an RA receptor to differentiate (Ikami *et al*., Development 2015, Tokue *et al*., Stem Cell Reports 2017).

SSCs compete for self-renewal factors in an open niche

We also question the mechanism that maintains a constant SSC density in the open niche of the testis. Evidence suggests that SSC density homeostasis is achieved through competing with each other for limited amounts of self-renewal factors (*e.g.*, FGFs). This mechanism was proposed as the "mitogen competition model" (Kitadate *et al*., Cell Stem Cell 2019).

Periodic differentiation of SSCs

Strikingly, differentiation of SSCs and subsequent differentiation to mature into sperm occur synchronously every 8.6 days. This is called the seminiferous epithelial cycle. We propose that the periodic differentiation is triggered by periodic synthesis and degradation of RA (Sugimoto *et al*., Mechanisms of Development 2012).

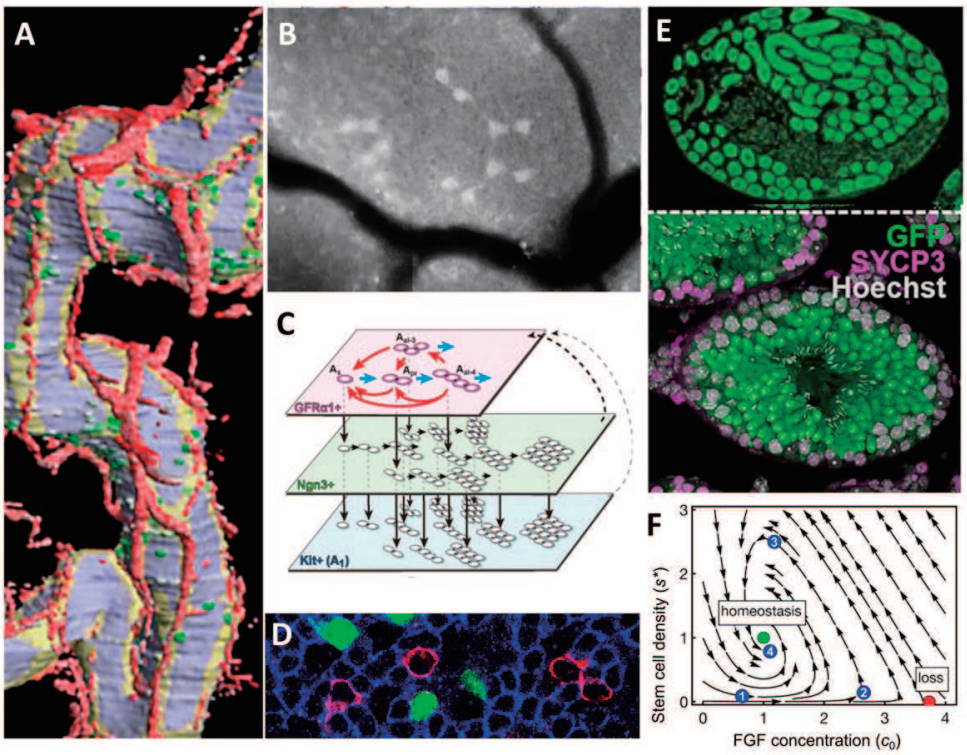


Figure 1. Different looks of mouse sperm stem cells (SSCs)
A. 3D-reconstructed seminiferous tubules, showing SSCs in green, blood vessels in red.
B. An intravital live-imaging filming of GFP$^+$ SSC syncytia undergoing differentiation.
C. Scheme of SSC dynamics in multi-compartment, interconverting singly-isolated and syncytial states.
D. Whole-mount immunofluorescence, showing SSCs (magenta) intermingled with primed (green) and committed (blue) progenies.
E. Sectioned host testis showing repopulated by transplanted donor SSCs (green), augmented by transient RA inhibition.
F. A phase portrait showing the dynamics of homeostatic SSC density regulation in the mitogen competition model.

Clonal analysis and tuning of post-transplantation SSC fate

When SSCs are transplanted into host animals' seminiferous tubules whose germ cells have been depleted, SSCs engraft and regenerate spermatogenesis (Brinster *et al*., 1994). What happens during repopulation was largely elusive. We found that donor SSCs follow stochastic fate with significant similarity with homeostasis. We further succeeded in tuning the donor SSC fate to increase the repopulation efficiency, which would promise the practical use of this technology (Nakamura *et al*., Cell Stem Cell 2021).

Identification of a tiny, most primitive fraction of SSCs

An important advance gained in FY2021 is that we have detailed gene expression heterogeneity of SSCs (precisely, a population called "undifferentiated spermatogonia") and their dynamical state transitioning in supporting the homeostatic and continual sperm production (Nakagawa *et al*., Cell Reports 2021). This is a collaborative study with Nagasawa (Osaka) and Simons (Cambridge) labs. We identified that a gene *Plvap* (plasmalemma vesicle associated protein) is expressed in a tiny, most primitive fraction of mouse SSCs (Figure 2A).

Detailing the heterogeneity of mouse SSCs

Plvap shows overlapping expression with some other genes, including Eomes, Pdx1, Shisa6, and T (brachyury). By dimension reduction of single-cell (sc)RNA-seq data (La *et al*., 2018), Plvap$^+$ cells cluster at the undifferentiated end of SSCs, forming a discrete subfraction of cells expressing established undifferentiated markers, GFRα1 and Id4. In contrast, differentiation-related genes (*e.g*., Sox3, Ngn3, and RARγ) is expressed in cells near the opposite end (Figure 2B). Based on these, Figure 2C summarizes the heteroge-

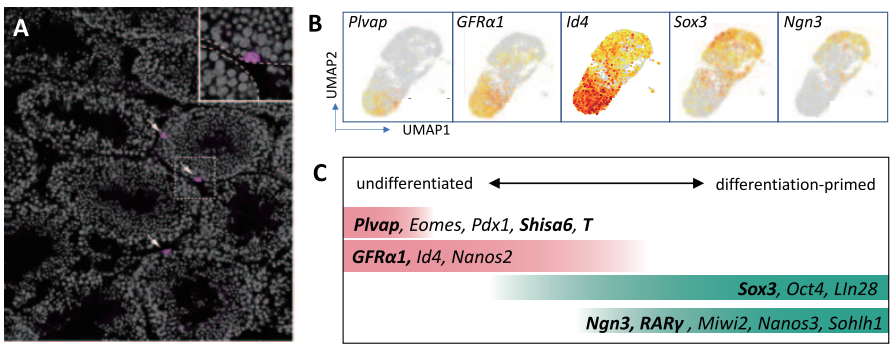


Figure 2. Heterogeneity of SSCs
A. Immunostaining of a mouse testis section for Plvap protein (magenta), with DNA counterstained in gray. Only three cells located on the periphery of seminiferous tubules express Plvap.
B. UMAP analyses of scRNA-seq data showing the expression of indicated genes.
C. A summary of expression profile of a set of genes within SSCs. Genes shown in bald have been analyzed in our lab.
Adopted from Cell Reports 37 (3), 109875.

neous expression of key SSC genes.

Contribution of multiple SSC fractions to self-renewing pool in homeostasis

By pulse-labeling using tamoxifen-dependent CreER, Plvap$^+$, Sox3$^+$, and Ngn3$^+$ cells were found to contribute to the self-renewing pool in homeostasis. In particular, after being sparsely labeled with GFP expression, these cells gave rise to cell patches comprised of GFP$^+$ germ cells in three months (Figure 3).

Interestingly, while the patch-forming efficiency varied between these fractions, the resultant individual patches showed indistinguishable size distribution irrespective of the original cell state. Further, strikingly, the same heterogeneous SSC population was established in every such patch, again, irrespective of the original cell state.

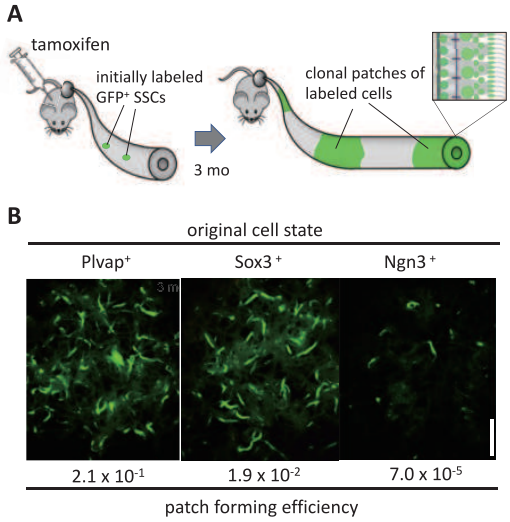


Figure 3. Pulse-labeling of multiple SSC fractions
A. Experimental design addressing the contribution of spermatogonia expressing particular genes to homeostasis, following a pulse-labeling by tamoxifen injection.
B. Results of the pulse-labeling of the Plvap$^+$, Sox3$^+$, and Ngn3$^+$ fractions. Note the differential patch forming efficiencies in contrast to the similarly-looking individual patches.

We reasoned that SSCs transit continually and reversibly between multiple cell states, from the self-renewal-biased Plvap$^+$ state to the differentiation-primed Ngn3$^+$ state (Figure 3B). As a result, a heterogeneous SSC pool is always established, which both maintains itself and gives rise to irreversibly committed progenitor cells of Kit$^+$ spermatogonia as a result of cell division and state transition.

A multistate dynamics: Proposal of an inclusive model of mouse SSCs

Finally, we synthesized a mathematical model that captures the multistate SSC dynamics in homeostasis. This model assumes that the cells undergo cell division and state transition (either differentiation and reversion) in a stochastic (*i.e.*, probabilistic) manner, following particular rates specific to each state (Figure 4).

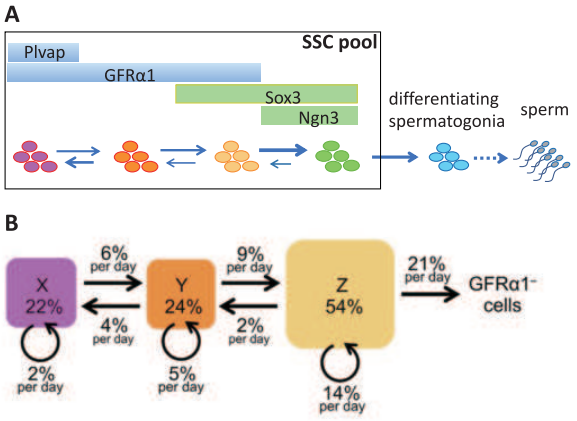


Figure 4. Multistate dynamics of mouse SSCs
A. A general perspective of the multistate dynamics, in which SSCs transit between multiple states reversibly to form a single SSC pool.
B. A numerically fit dynamics of mouse SSCs in homeostasis. The flows of cells between cell states and cell division at respective states are shown in values relative to the total GFRα1$^+$ cells per day. Note that the GFRα1$^-$ (Ngn3$^+$) to GFRα1$^+$/Sox3$^+$ state is not defined due to its low frequency in this model. Adopted from Cell Reports 37 (3), 109875.

Interestingly, such a multistate dynamics is found capable of saving the cell divisions in the self-renewing pool in producing a certain number of differentiating cells. This is due to the differential cell division frequencies between the states; the more primitive the state is, the less frequently the SSC divide. See Nakagawa *et al*., Cell Reports 2021 for detail.

Furthermore, this quantitative model can explain the seemingly contradictory models proposed for the SSC dynamics, and may provide inclusive insights into this long-held problem in the field of SSC research.

Publication List:

〔Original Papers〕

- Nakagawa, T., Jörg, D.J., Watanabe, H., Mizuno, S., Han, S., Ikeda, T., Omatsu, Y., Nishimura, K., Fujita, M., Takahashi, S., Kondoh, G., Simons, B.D., Yoshida, S., and Nagasawa, T. (2021). A multistate stem cell dynamics maintains homeostasis in mouse spermatogenesis. Cell Rep. *37*, 109875. DOI: 10.1016/j.celrep.2021.109875
- Nakamura, Y., Jörg, D.J., Kon, Y., Simons, B.D., and Yoshida, S. (2021). Transient suppression of transplanted spermatogonial stem cell differentiation restores fertility in mice. Cell Stem Cell *28*, 1443-1456. e7. DOI: 10.1016/j.stem.2021.03.016
- Osawa, Y., Murata, K., Usui, M., Kuba, Y., Le, H.T., Mikami, N., Nakagawa, T., Daitoku, Y., Kato, K., Shawki, H.H., Ikeda, Y., Kuno, A., Morimoto, K., Tanimoto, Y., Dinh, T.T.H., Yagami, K., Ema, M., Yoshida, S., Takahashi, S., Mizuno, S., and Sugiyama, F. (2021). EXOC1 plays an integral role in spermatogonia pseudopod elongation and spermatocyte stable syncytium formation in mice. eLife *10*, e59759. DOI: 10.7554/eLife.59759

〔Review article〕

- Kitadate, Y., and Yoshida, S. (2022). Regulation of spermatogenic stem cell homeostasis by mitogen competition in an open niche microenvironment. Genes Genet. Syst. *97*, 15–25. DOI: 10.1266/ggs.21-00062

Laboratory of Regeneration Biology

Director General
AGATA, Kiyokazu

Specially Appointed Associate Professor: *SUZUKI, Ken-ichi*
Postdoctoral Fellow: *TERAMOTO, Machiko*
SOKENDAI Graduate Student: *SUGIURA, Nao*
KUROKI, Yoshihito
BO, Kazuto
Visiting Graduate Student: *ISHIDA, Miyuki*
Technical Assistant: *KAJIURA-KOBAYASHI, Hiroko*
Admin Support Staff: *SAKAGAMI, Mari*
NISHIMURA, Noriko

Comparative Regenerative Biology

We use animals that demonstrate a high ability in regenerating body parts, 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 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), and accomplishing functional joint regeneration in frogs through the activation of reintegration systems (Tsutsumi *et al*., 2016 Regeneration). We are currently trying to induce functional joint regeneration in mouse and to characterize adult pluripotent stem cells in planarian at a single cell level.

Trials for evoking functional joint regeneration in mouse

Mouse can't regenerate joint structures at all. However, we expect that functional joints might be evoked by the activation of reintegration systems since we found that the remaining tissues after amputation at the joint level possessed the ability to regenerate joint tissues (Hotta *et al*., unpublished data). That is, the mouse might lose their regenerative abilities due to non-cell autonomous reasons. Therefore, we are now investigating how to activate reintegration systems in mice after amputation at the joint level for evoking functional joint regeneration.

Isolation of the viable planarian aPSC (adult pluripotent stem cells) and characterization of these cells at the single cell level.

We tried to develop an isolation method for viable adult pluripotent stem cells (aPSC) from planarians using FACS. In the previous method, isolated aPSC were unhealthy after staining with several fluorescence dyes. Recently, we found a condition to be able to isolate viable aPSC by FACS sorting without staining with dyes. The single cell transcriptome analyses using these isolated aPSC showed unique properties of planarian aPSC (Kuroki *et al*., unpublished).

We also found that these aPSC showed the collective migration property (branch-like patterns in Fig.1) after RNAi treatment of the *MTA* (*Metastatic Tumor Antigen*) family genes, suggesting that planarian might have unique niche for maintain aPSC in the adult bodies (Sato *et al*., 2022).

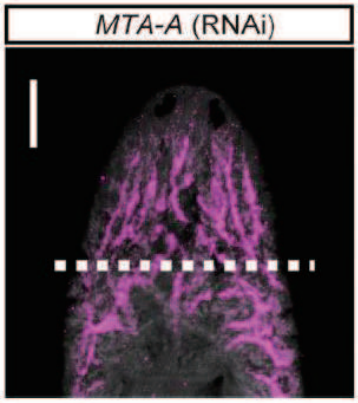


Figure.1 RNAi treated animal of the *MTA-A* gene showed the branch-like distribution patterns of aPSC (magenta stained with the PIWI-A antibodies).

Now, we are investigating differences of these cellular systems between highly- and lower- regenerative animals.

Publication List:

〔Original papers〕

- Bando, T., Okumura, M., Bando, Y., Hagiwara, M., Hamada, Y., Ishimaru, Y., Mito, T., Kawaguchi, E., Inoue, T., Agata, K., Noji, S., and Ohuchi, H. (2022). Toll signalling promotes blastema cell proliferation during cricket leg regeneration via insect macrophages. Development *149*, dev199916. DOI: 10.1242/dev.199916
- Finet, C., Kassner, V.A., Carvalho, A.B., Chung, H., Day, J.P., Day, S., Delaney, E.K., De Re, F.C., Dufour, H.D., Dupim, E., Izumitani, H.F., Gauterio, T.B., Justen, J., Katoh, T., Kopp, A., Koshikawa, S., Longdon, B., Loreto, E.L., Nunes, Maria D.S., Raja, K.K.B, Rebeiz, M., Ritchie, M.G., Saakyan, G., Sneddon, T., Teramoto, M., Tyukmaeva, V., Vanderlinde, T., Wey, E.E., Werner, T., Williams, T.M., Robe, L.J., Toda, M.J., and Marletaz, F. (2021). DrosoPhyla: Resources for Drosophilid Phylogeny and Systematics. GENOME Biol. Evol. *13*, evab179. DOI: 10.1093/gbe/evab179
- Inoue, T., and Agata, K. (2022). Quantification of planarian behaviors. Dev. Growth & Differ. *64*, 16–37. DOI: 10.1111/dgd.12765
- Lee, H., Hikasa, K., Umesono, Y., Hayashi, T., Agata, K., and Shibata, N. (2022). Loss of plac8 expression rapidly leads pluripotent stem cells to enter active state during planarian regeneration. Development *149*, dev199449. DOI: 10.1242/dev.199449
- Sato, Y., Umesono, Y., Kuroki, Y., Agata, K., and Hashimoto, C. (2022). Proliferation maintains the undifferentiated status of stem cells: The role of the planarian cell cycle regulator Cdh1. Dev. Biol. *482*, 55–66. DOI: 10.1016/j.ydbio.2021.12.006
- Takeuchi, T., Matsubara, H., Minamitani, F., Satoh, Y., Tozawa, S., Moriyama, T., Maruyama, K., Suzuki, K.T., Shigenobu, S., Inoue, T., Tamura, K., Agata, K., and Hayashi, T. (2022). Newt Hoxa13 has an essential and predominant role in digit formation during development and regeneration. Development *149*, dev200282. DOI: 10.1242/dev.200282

DIVISION OF BEHAVIORAL NEUROBIOLOGY

Professor
HIGASHIJIMA, Shin-ichi

Assistant Professor:	*KIMURA, Yukiko*
	TANIMOTO, Masashi
Technical Staff:	*TAKEUCHI, Yasushi*
SOKENDAI Graduate Student:	*SUGIOKA, Takumi*
	KAWANO, Kohei
	SHIMIZU, Sayaka
Visiting Undergraduate:	*KATAYAMA, Taisei*
Technical Assistant:	*WATAKABE, Ikuko*
	ITO, Hiroko
	TAKEUCHI, Yoshiko
	SETO, Kumiko

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).

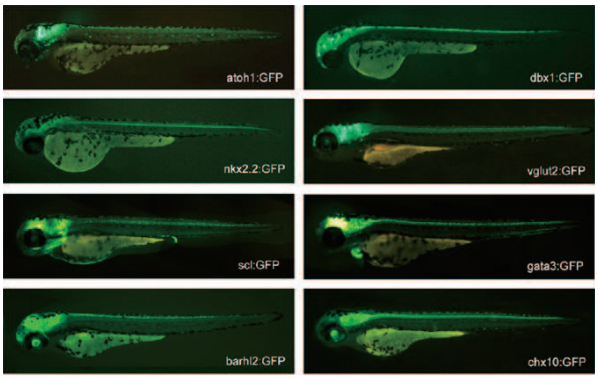


Figure 1. Examples of transgenic fish expressing GFP in specific classes of neurons.

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. Long descending commissural V0v neurons ensure coordinated swimming movements along the body axis in larval zebrafish

In the early developmental stage, most animals can only exhibit immature forms of behaviors. As development progresses, they acquire the ability to produce more mature or refined forms of behaviors. Concurrent with such changes, many new connections are formed in the nervous system, which suggests that the formation of new connections is linked to the developmental maturation of behaviors. In animals in which new neurons are generated during development (*e.g.*, fish and amphibians), the incorporation of new neurons into the pre-existing circuits together with the forming of new connections likely contributes to the maturation of movements.

One example of this developmental maturation of behaviors is seen in the swimming behavior of larval zebrafish. Older larvae (*i.e.*, 4–5 dpf) exhibit more refined forms of swimming than younger larvae (*i.e.*, 2 dpf), During this period (from 2 dpf to 4–5 dpf), new neurons are generated in both the brain and spinal cord, with the latter being mainly responsible for generating swimming outputs. One class of premotor spinal neurons that are added later to the early spinal neuronal circuits are MCoD neurons, a subclass of V0v neurons (V0v neurons represent an excitatory class of neurons derived from the p0 developmental domain of the spinal cord). MCoD neurons are absent in the early stages and develop in a later phase of neurogenesis in the spinal cord. MCoD neurons are active during slow swimming. As for their function, one study showed that they contribute to the general excitability of spinal swimming circuits, and their ablation decreased the occurrence frequency of spontaneous swimming. Their function in the more specific aspect of slow swimming remains elusive, however.

One of the most characteristic features of slow swimming in older larvae is the stability of the head in the yaw dimension. In slow swimming, the muscle contractions are mostly confined to the trunk that is caudal to the swim bladder. Given that the center of the mass is located near the swim bladder in larval zebrafish (Figure 2A), it is thought that the head yaw displacement is produced by the recoil of the yawing moment force generated in the trunk. Considering this, the stability of the head yaw indicates that the net yawing moment force in the trunk that acts to the center of the mass is very small during slow swimming. Swimming consists of a descending wave of muscle contraction along the trunk. With this movement, the bending of the body transmits force to the surrounding water, and this region of the body, in turn, receives reaction force. To make the net yawing moment force minimal, the movements of the rostral and caudal parts of the trunk need to be highly coordinated with the diagonal dimension; when the rostral part receives leftward force, for example, the caudal part needs to receive rightward force. For this to occur, the swimming body form cannot be C-shaped (unilateral body bend); rather, the shape needs to be sinusoidal (S-shaped). MCoD neurons are a good candidate for implementing this coordinated movement of the trunk in the diagonal dimension, because they are active during slow swimming, and because they are long-distance descending commissural excitatory neurons that make direct connections onto MNs in the caudal region of the contralateral spinal cord (Figure 2B).

In this study, we tested whether MCoD neurons play a role in the coordinated movements of the trunk in the diagonal dimension, thereby ensuring minimal head yaw displacement during slow swimming. Our laser ablation experiments revealed that MCoD neurons do indeed play the expected role. In the MCoD-ablated larvae, the normal S-shaped body form during swimming was often lost with increased appearance of unilateral C-shaped bends. Concurrently, the head yaw stability was greatly impaired (Figure 3). In addition to swimming, the present study also sheds light on the evolutionarily conserved role of V0v neurons. In mice, long-distance descending commissural V0v neurons have been implicated in interlimb coordination during walking in the diagonal dimension[18]. We suggest that the long-distance descending commissural V0v neurons for the coordinated movements of the body/limbs in the diagonal dimension are the evolutionarily conserved pathway in spinal locomotor circuits.

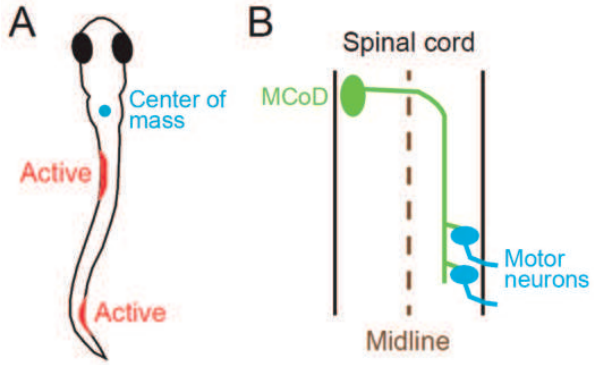


Figure 2. (A) One of the swim forms of a zebrafish larva at 4 to 5 dpf. The cyan circle shows the center of mass, which is located near the swim bladder. Muscle contractions are presumed to occur in the two locations marked in red. (B) Projection pattern of an MCoD neuron in the spinal cord. The axon of the MCoD neuron crosses the midline (broken line), descends on the contralateral spinal cord, and makes mono-synaptic excitatory connections onto caudally located MNs.

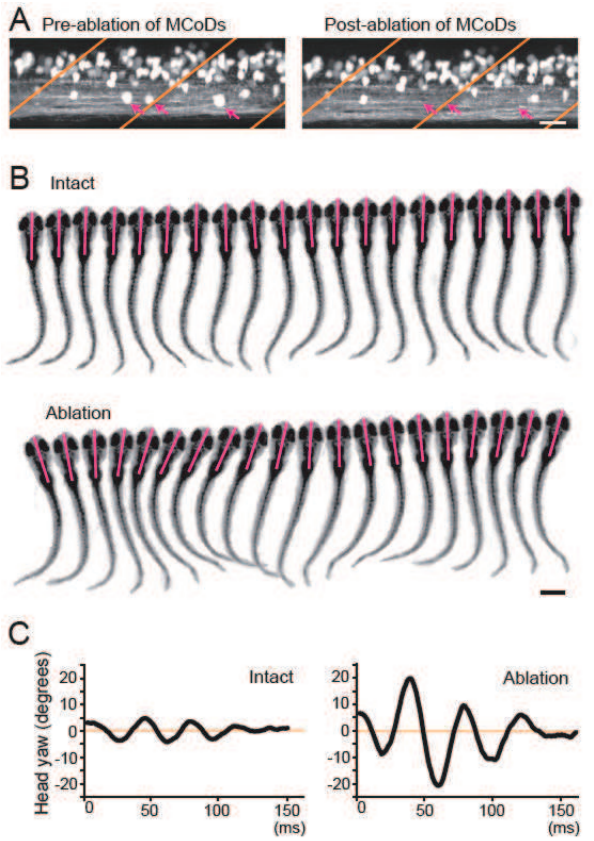


Figure 3. (A) Confocal stacked images of Tg[*evx*2-hs:GFP] fish before (left) and after (right) laser ablation. Images of two hemi-segments are shown. Magenta arrows show MCoD neurons that were chosen for laser ablation. MCoD neurons can be identified by their very ventral location in the spinal cord. Brown lines show boundaries of muscle segments. Scale bar, 20 μm. (B) Successive images captured at 1000 frames per second of larval zebrafish swimming. Images of every three frames (3 ms interval) are shown. Magenta bars depict the head directions in each frame. Top, images of an intact fish. Bottom, images of an MCoD-ablated fish. Scale bar, 500 μm. (C) Graphs of head yaw angle (y axis) versus time (x-axis) during swimming. Left, intact fish. Right, MCoD-ablated fish.

III. *In vivo* functional imaging analysis of the vestibular sensory organ

Maintaining head and body orientation relative to the Earth's vertical gravity axis is vital for survival. Vestibular organs in the inner ear play a crucial role for this task. Sensory hair cells in the otolith organs receive linear acceleration, *e.g.*, head tilt, translation and vibration. Direction of the acceleration is detected by the polarized arrangement of hair bundles in the hair cells (Figure 4). Each otolith organ contains hair cells with different but topographically organized hair-bundle polarity that reverses at a line of polarity reversal (LPR). Although morphofunctional specialization of the vestibular hair cells has been widely studied, the direction- and modality-selective responses to the head motion have not been systematically studied *in vivo*, therefore how the head motion signals are processed in the vestibular system remains unclear.

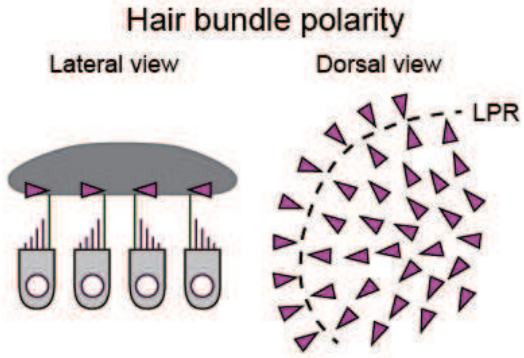


Figure 4. Hair bundle polarity (arrowheads) in the utricle.

To visualize hair cell responses to the head motion, we built a microscope in which an objective lens can tilt with a small sample 360 degree by a motorized stage during Ca^{2+} imaging (Figure 5). A spinning-disk confocal scanner and an image splitting optics formed green and red fluorescent images on a digital camera. This ratiometric imaging setup reduced artifacts derived from non-uniformity of the excitation light and optical distortion during the optics motion. With this tiltable objective microscope, we imaged neural activity in all the utricular hair cell at the single-cell level during pitch or roll tilt/vibration in 5-day-old transgenic zebrafish larvae expressing Ca^{2+} indicator, jGCaMP7f, and red fluorescent protein, tdTomato, in the hair cells.

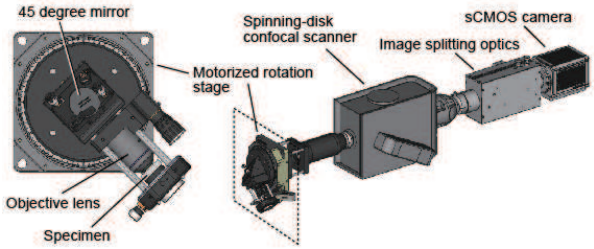


Figure 5. Tiltable objective microscope.

Consistent with the morphological hair-bundle polarity, hair cells medial to the LPR are activated by the lateral-down roll tilts, whereas those lateral to the LPR are activated by the medial-down tilts (Figure 6). In response to the nose-down pitch tilts, hair cells medial to the LPR in the rostral utricle and those lateral to the LPR in the caudal utricle are activated, whereas the rest of the hair cells are activated by the tail-down tilts. Interestingly, hair cells in the medial utricle exhibited larger responses to the head tilt compared to the lateral hair cells. In contrast to the responses to the head tilt, the vibratory stimulus in the pitch or roll axis activated the hair cells only in the rostral and lateral utricle near the LPR.

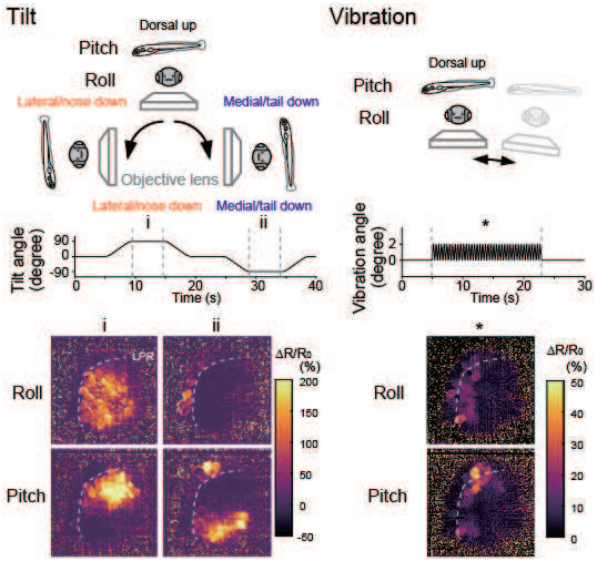


Figure 6. Hair cell responses to head tilt or vibration in the utricle

Together, the tiltable objective microscope visualized, for the first time to our knowledge, the topographically organized response selectivity for the stimulus direction and modality in the vestibular periphery. The imaging strategy we have established here is applicable to the central nervous system, and thus it will provide deeper understanding of the vestibular processing in the brain.

Publication List:

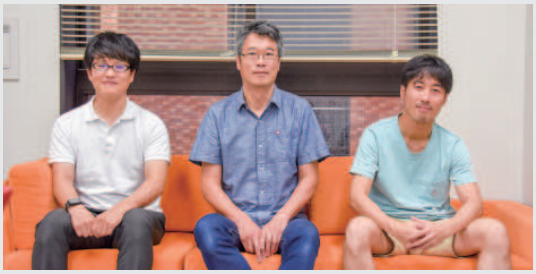
〔Original Papers〕

- Bohm, U.L., Kimura, Y., Kawashima, T., Ahrens, M.B., Higashijima, S., Engert, F., and Cohen, A.E. (2022). Voltage imaging identifies spinal circuits that modulate locomotor adaptation in zebrafish. Neuron *110*, 1211+. DOI: 10.1016/j.neuron.2022.01.001
- Kawano, K., Kato, K., Sugioka, T., Kimura, Y., Tanimoto, M., and Higashijima, S. (2022). Long descending commissural V0v neurons ensure coordinated swimming movements along the body axis in larval zebrafish. Sci. Rep. *12*, 4348. DOI: 10.1038/s41598-022-08283-0
- Suzuki, D.G., Wada, H., and Higashijima, S. (2021). Generation of knock-in lampreys by CRISPR-Cas9-mediated genome engineering. Sci. Rep. *11*, 19836. DOI: 10.1038/s41598-021-99338-1

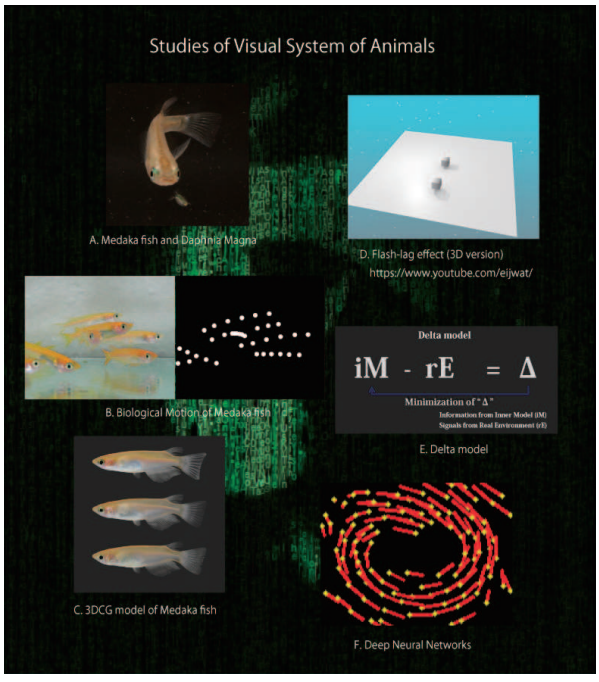
LABORATORY OF NEUROPHYSIOLOGY

Associate Professor
WATANABE, Eiji

Specially Appointed Assistant Professor: *KOBAYASHI, Taisuke*
JSPS Postdoctoral Fellow: *NISHIUMI, Nozomi*
Visiting Scientist: *KOMATSU, Hidehiko*
Technical Assistant: *WATABE, Mihoko*
TSURUMARU, Mayumi
SUGINAGA, Tomomi



We believe that the best way to understand mechanisms underlying a certain system is to reconstruct the system as it exists. By utilizing 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 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. As a result, 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 (Matsunaga & Watanabe, 2012). As a side-story, we analyzed the swimming behaviors of adult water flea *Daphnia magna*, and found apparent sexual differences: laterally biased diffusion of males in contrast to the nondirectional diffusion of females (Toyota *et al*., 2022).

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).

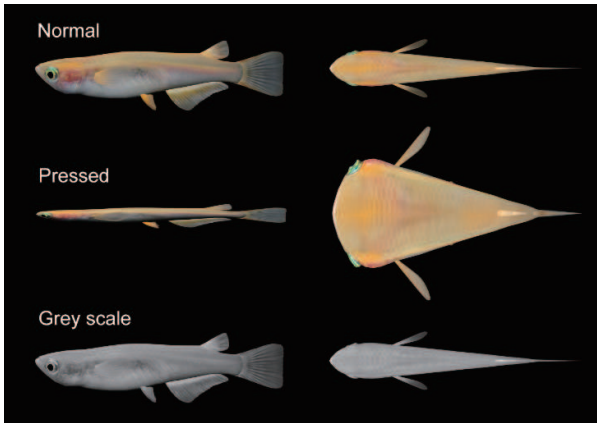


Figure 1. Virtual medaka fish.

II. Psychophysical study of human & AI vision

Another of our major subjects is the psychophysical and theoretical studies of the visual illusions experienced by human and artificial intelligence (AI). 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 novel visual illusions, such as the shelf-shadow illusion (3rd Award of the Illusion Contest), the Monstre Benham illusion (2nd Award), the Morning Monster Illusion (10th Award), and the Genkikan Illusion (Figure 2).

Figure 2. Okazaki Genkikan.

Watanabe (NIBB) and Sinapayen (Sony CSL) got 2nd place at the 13th Visual Illusion Contest, for the Okazaki Genkikan Illusion. It is one of the Poggendorff illusions. (http://www.psy.ritsumei.ac.jp/~akitaoka/sakkon/sakkon2021.html). It was found in Okazaki Genkikan Facility.

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. (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) (Evolutionary Generation of Visual Motion Illusions, https://arxiv.org/abs/2112.13243, Sinapayen and Watanabe, 2021). Currently, a variety of research is being conducted using these AI systems (https://arxiv.org/abs/2106.12254).

In 2022, we examined the properties of the network using a set of 1500 images, including ordinary static images of paintings and photographs and images of various types of motion illusions (Visual Illusions Dataset, https://doi.org/10.6084/m9.figshare.9878663). Results showed that the networks clearly classified a group of illusory images and others and reproduced illusory motions against various types of illusions similar to human perception. Notably, the networks occasionally detected anomalous motion vectors, even in ordinally static images where humans were unable to perceive any illusory motion. Additionally, illusion-like designs with repeating patterns were generated using areas where anomalous vectors were detected, and psychophysical experiments were conducted, in which illusory motion perception in the generated designs was detected (Figure 3). The observed inaccuracy of the networks will provide useful information for further understanding information processing associated with human vision (Kobayashi *et al*., 2022)

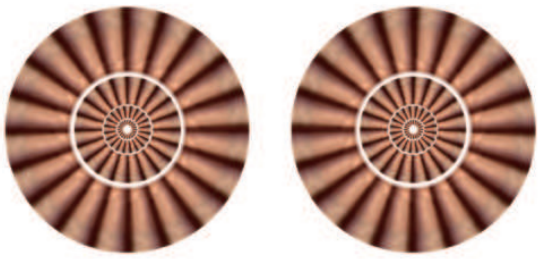


Figure 3. A visual illusions extracted by AI

III. Ecological study of tactics in predators and prey.

We are interested in behavioral interactions between predators and prey concerning the ecological aspect. In the process of the co-evolution, predators and prey have developed various tactics to overcome each other. To elucidate the sophistication of these tactics, we have examined the mechanisms and efficacies of predatory and antipredator behaviors of several animals such as fish, dragonflies, bats and birds. In addition, we have developed experimental system for those studies, which present interactive virtual predators to prey animals based on animation (Figure 4) or robotics. This experimental system consists of three modules as a sensing, a command generating and a virtual animal module. According to the spatial scale, the system employs appropriate technologies in each module. For example, in a small aquarium scale, we use high speed cameras as a sensing module and virtual reality animation as a virtual animal module. On the other hand, in a large outdoor scale, we use real-time GPS as a sensing module and unmanned aerial vehicle as a virtual animal module. Therefore this system are able to cover wider range of animals and expected to strongly support the study of animal interactions.

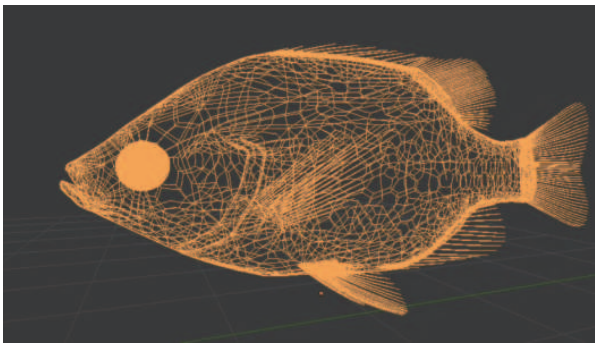


Figure 4. A computer animation of the virtual predatory fish.

Publication List:

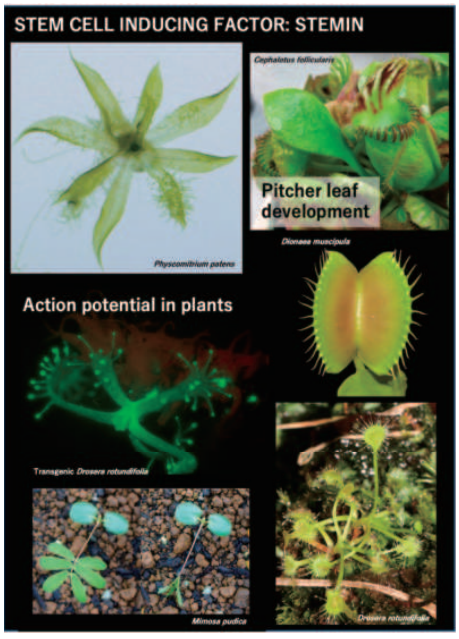
〔Original paper〕

● Kobayashi, T., Kitaoka, A., Kosaka, M., Tanaka, K., and Watanabe, E. (2022). Motion illusion-like patterns extracted from photo and art images using predictive deep neural networks. Sci. Rep. *12*, 3893. DOI: 10.1038/s41598-022-07438-3

DIVISION OF EVOLUTIONARY BIOLOGY

Professor
HASEBE, Mitsuyasu

Assistant Professor:	*ISHIKAWA, Masaki*
	SEGAMI, Shoji
Specially Appointed Assistant Professor:	
	MANO, Hiroaki
	KOSETSU, Ken
Technical Staff:	*OHI, Shoko*
Postdoctoral Fellow:	*NARUKAWA, Hideki*
	PALFALVI, Gergo
SOKENDAI Graduate Student:	*HORIUCHI, Yuta*
	DE VILLIERS, Ruan
	CHEN, Peng
	UEDA, Masamichi
Visiting Graduate Student:	*TANASE, Kuniaki*
Visiting Undergraduate:	*MATSUDA, Riku*
Technical Assistant:	*AOKI, Etsuko*
	FUKADA, Hatsumi
	HIRAMATSU, Mika
	KAJIKAWA, Ikumi
	MATSUZAKI, Yoko
	MASUOKA, Tomoko
	NISHI, Tayo
	ZHANG, Liechi
	OGAWA, Yuko
	NAGATA, Kyoko
	SAITO, Yuki
	ASAOKA, Mayumi
	BUSHRA, Samira
Admin Support Staff:	*KOJIMA, Yoko*
	HASEBE, Yuki



I. Evolution of Complex Adaptive Characters

The theory of natural selection and the neutral theory of molecular evolution are fundamental concepts in evolutionary biology. However, even with such theories, there still remain unexplained phenomena, one of which is the evolution of complexity. It is difficult to explain the mechanisms needed to evolve complex adaptive traits, because such traits comprise plural components and become adaptive only when all components are gathered together. However, based on evolutionary theories, each component should evolve one by one according to the accumulation of mutations.

To understand the molecular and cellular bases of adaptive traits, we study the molecular and cellular mechanisms and evolution of (1) long distance signaling in plants, (2) reprogramming from differentiated cells to stem cells, and (3) development and morphology in land plants.

II. Molecular mechanisms and evolution of long distance signaling in plants

Plants lack blood flow and nerves, but have evolved unique long-distance intercellular signaling mechanisms. Signals using plant hormones, peptides, proteins, and slow calcium waves have been well elucidated; however, the molecular mechanism of long-range, rapid, intercellular signaling by action potentials with fast calcium waves, which evolved in parallel to similar signaling mechanisms in animals, remains largely unknown. Rapid transmission of action potentials has been reported in specific tissues of the sensitive plant *Mimosa pudica*, the Venus flytrap *Dionaea muscipula*, and the sundew *Drosera rotundifolia*. We use these species, as well as *Arabidopsis thaliana*, to study the molecular mechanisms and evolution of action potential generation and transmission. We have obtained genome sequences for these species (Palfalvi *et al*. 2020 Curr. Biol. for *Dionaea* and unpublished for *Mimosa pudica* and *Drosera rotundifolia*) and established techniques for transformation (Mano *et al*. 2014 PLoS ONE for *Mimosa pudica*; Suda *et al*. 2020 Nat. Plants for *Dionaea*; unpublished for *Drosera rotundifolia*). We intend to screen for genetic factors responsible for fast intracellular and intercellular electrical signaling by action potentials, analyze their molecular characteristics such as effects on ion permeability and intracellular localization, and perform genetic gain- and loss-of-function experiments. This will allow us to understand the general mechanisms of action potential transmission in plants and the evolutionary process that resulted in diversity of transmission velocity, which is adaptive in the three plants.

II-1. Molecular mechanisms of long distance signaling of mechano-stimuli in the sensitive plant *Mimosa pudica*

The sensitive plant *Mimosa pudica* folds it leaves within a couple of seconds in response to touch. These rapid movements are mediated by specialized motor organs, pulvini, which locate at the base of each compound leaves, pinnae, and small leaflets. When getting stimulated, motor cells in one side of the pulvinus (extensor half) shrink while cells in the opposite side (flexor half) do not shrink, consequently leading to the unidirectional bending of the pulvinus. Because

a pulvinus contains thousands of motor cells, achievement of the rapid movement at a whole pulvini requires not only the rapid shrinkage of individual motor cells but also rapid synchronization mechanisms among them. The molecular mechanisms underlying these rapid shrinkage and rapid cell-cell communication still remain open questions.

To elucidate the molecular mechanisms for rapid leaf movements, we have developed an efficient transformation technique in *M. pudica*, and then established *in planta* live imaging of Ca^{2+} with sensor fluorescent proteins and CRISPR/Cas9-mediated gene knockout. To obtain candidate genes responsible for rapid movements, we compared gene expression profiles between pulvini and non-motor organs, and between extensor and flexor halves of pulvini. As a result, we got a number of extensor-enriched genes. After the systematic knockout experiments of these candidate genes, mutants of two channel genes, one gene encoding a membrane-associated protein with uncharacterized functions, and one transcription factor showed deficiencies in touch-induced rapid movements. Currently we are working on the detailed analysis on functions of those genes and mutans by using transgenic techniques and electrophysiological studies employing a heterologous expression system in *Xenopus* oocytes.

Rapid movements in *M .pudica* take only 1 to 2 seconds for the whole process and our preliminary observations suggested that their initial events like Ca^{2+} increase occurred within 100 ms or less. To achieve higher time resolution for our fluorescent live imaging, we introduced a high-speed and high-sensitivity camera system, and developed a method to analyze spatio-temporal propagation of Ca^{2+} signals robustly. We found that in the tertiary pulvinus, a mechanical stimulus applied at the center evokes a rapid concentric propagation of Ca^{2+} wave with 1.5 cm/s velocity, and it only took 30 ms to reach to the outermost edge of excitable motor cells in the extensor half (Figure 1). These analytical methods will facilitate future studies using mutant lines described above. This study was mainly performed by Dr. Hiroaki Mano with the support by technical staff.

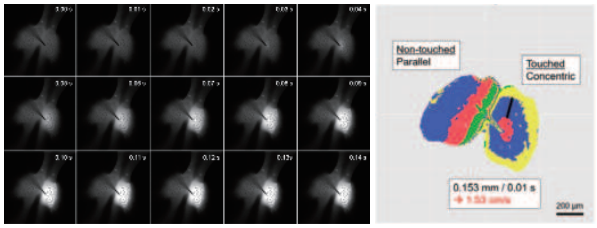


Figure 1. Fluorescence images of a red Ca^{2+} sensor R-GECO in a *M. pudica* tertiary pulvinus after stimulated with a needle (100 fps). Right image shows how the wave front propagates. 4 colors are repeatedly used (R-B-Y-G) to discriminate each 10 ms frame.

II-2. Molecular mechanisms and evolution of the generation and transmission of action potential in the sundew *Drosera rotundifolia* and the Venus flytrap *Dionaea muscipula*

Drosera rotundifolia is a carnivorous plant that captures prey for nutrients by its tentacles, multicellular hairs on the leaf with sticky digestive fluid at their heads. Each tentacle senses mechano- and chemical-stimuli at its head, and cells in a tentacle base bend to bring the captured prey to the center of the trap leaf. In this process, action potential is elicited at the head by the stimulus and transmits through the stalk cells to the basal cells. The advantage of *D. rotundifolia* to study transmission mechanisms of action potential is in the simple structure of the tentacle, which is feasible for electrophysiology and bio-imaging. We succeeded to cultivate *D. rotundifolia* under aseptic conditions with proper reactions to the stimuli, and furthermore, to transform *D. rotundifolia* with the green fluorescent protein gene. We improved the transformation efficiencies with optimizing nutrient conditions in the medium.

In this fiscal year, nuclear genome of *D. rotundifolia* was sequenced with long-read sequencing with Nanopore sequencer and linked-read sequencing with TELL-seq library and Illumina sequencer in 50 times coverage. To look for candidate genes to function in the generation and transmission of action potential, we compared mRNA profiles between different tissues in tentacles and between tentacles and laminas. Loss-of-function experiments with CRISPR/Cas9 mediated gene knockout are ongoing.

Ca^{2+} sensor RGECO expressed transformants were established to investigate the relationship between rapid calcium wave and action potential transmission. Mechanical stimulation to the tentacle head produced a fast Ca^{2+} wave with similar speed to the action potential, which was transmitted to the basal cells, but not to laminar cells. We further investigate the molecular and cellular mechanisms with other transgenic lines.

The Venus flytrap is another carnivorous plant, whose leaf blade bends to capture prey with two successive mechanical stimuli applied to the sensory hairs on the surface. The mechanical stimulus is sensed at the middle cells of the hair, in which action potential is generated to transmit to whole laminar cells. As done in *D. rotundifolia*, we looked for candidate genes with comparative transcriptomics, and gene knockout experiments are ongoing. This study was mainly conducted by Dr. Shoji Segami, Ms. Shoko Ooi, Mr. Peng Chen, Mr. Kuniaki Tanase, and Mr. Riku Matsuda with a the support of technical staff. This work is collaboration work with Drs. Hiraku Suda and Masatsugu Toyota in Saitama University.

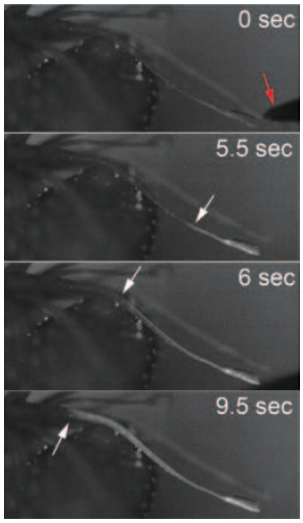


Figure 2. Fluorescence images of a RGECO *Drosera rotundifolia* tentacle after the tip was stimulated with a needle (red arrow in 0 sec), showing changes of cytosolic calcium ion amounts. White arrows indicate fronts of calcium waves.

III. Molecular mechanisms of reprogramming from differentiated cells to stem cells by STEMIN

Stem cells self-renew and give rise to differentiated cells to form tissues or organs. Differentiated cells in land plants can be readily reprogrammed into stem cells during post-embryonic development and regeneration. Thus, land plants have an intrinsic ability to initiate a new genetic regulatory network, despite the existence of epigenetic memory in differentiated cells. The underlying molecular mechanism and the changes in the network due to mutation in its components contribute to the environmental adaptability of land plants to survive under harsh environments and to the evolution of complex adaptive traits.

To understand the molecular basis of reprogramming, we have used the moss *Physcomitrium patens*, in which leaf cells can be reprogrammed into chloronema apical stem cells by wounding. During this process, the expression of *STEMIN1* gene, which encodes AP2/ERF transcription factor, is activated at leaf cells facing the cut. Deletion of *STEMIN1* and its two homologs retards reprogramming, while ectopic *STEMIN1* induction changes differentiated cells into chloronema apical stem cells. We also found that transient DNA damage induces reprogramming of leaf cells into stem cells, which requires the expression of *STEMIN* genes. Thus, *STEMIN* genes play a role in both the wounding- and the DNA damage-induced cellular reprogramming (Figure 3).

We found that ectopic induction of STEMIN1 in leaf cells decreases a repressive chromatin mark, histone H3 lysine 27 trimethylation (H3K27me3), on its direct target genes. To elucidate the mechanism regulating the local H3K27me3 reprogramming, we screened for STEMIN1-interacting proteins and found that some of the components have the potential to function in the histone modification. These suggest that STEMIN1 could have a role as a platform to recruit components needed for local H3K27me3 reprogramming at the specific loci of the genome. This study was mainly performed by Dr. Masaki Ishikawa and Mr. Ruan De Villiers with the support of the technical staff.

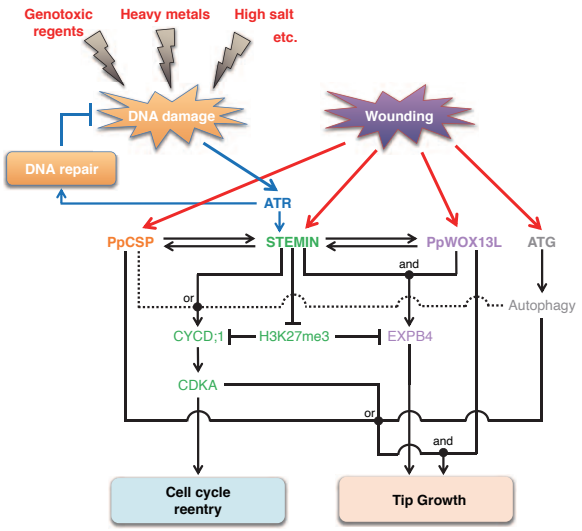


Figure 3. A working hypothesis of a genetic regulatory network of cellular reprogramming in *Physcomitrium patens*.

IV. Evolution of Development and morphology in land plants

Expansion of individual cells contributes to the morphogenesis of multicellular organisms, especially in land plants, in which cell migration is restricted by the rigid cell wall. Cell walls are mainly composed of the polysaccharides and the outer lipid cuticle layer. Previous reports showed that mechanisms acting on the cell wall polysaccharides, such as cellulose and pectin, regulate cell expansion. However, how other factors regulate cell expansion is not clear. We found that an ATP-binding cassette (ABC) B family transporter, PpABCB14, localized on the plasma membrane of the expanding region of gametophore initial cells and the leaf cells. Mr. Liechi Zhang and Dr. Masaki Ishikawa mainly investigate the molecular mechanisms with support of the technical staff.

Publication List:

〔Original papers〕

- Bao, L., Inoue, N., Ishikawa, M., Gotoh, E., Teh, O.-K., Higa, T., Morimoto, T., Ginanjar, E.F., Harashima, H., Noda, N., Watahiki, M., Hiwatashi, Y., Sekine, M., Hasebe, M., Wada, M., and Fujita, T. (2022). A PSTAIRE-type cyclin-dependent kinase controls light responses in land plants. Sci. Adv. *8*, eabk2116. DOI: 10.1126/sciadv.abk2116
- Kamada, T., Otomo, K., Murata, T., Nakata, K., Hiruma, S., Uehara, R., Hasebe, M., and Nemoto, T. (2022). Low-invasive 5D visualization of mitotic progression by two-photon excitation spinning-disk confocal microscopy. Sci. Rep. *12*, 809. DOI: 10.1038/s41598-021-04543-7
- Kanne, J. V, Ishikawa, M., Bressendorff, S., Ansbol, J., Hasebe, M., Rodriguez, E., and Petersen, M. (2021). Overexpression of ATG8/LC3 enhances wound-induced somatic reprogramming in *Physcomitrium patens*. Autophagy. *18*, 1463-1466.DOI: 10.1080/15548627.2021.1975913
- Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., and Shigenobu, S. (2021). Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*. eLife *10*, e60176. DOI: 10.7554/eLife.60176
- Plant Cell Atlas Consortium, Jha, S.G., Borowsky, A.T., Cole, B.J., Fahlgren, N., Farmer, A., Huang, S.-S.C., Karia, P., Libault, M., Provart, N.J., Rice, S.L., Saura-Sanchez, M., Agarwal, P., Ahkami, A.H., Anderton, C.R., Briggs, S.P., Brophy, J.A., Denolf, P., Di Costanzo, L.F., Exposito-Alonso, M., Giacomello, S., Gomez-Cano, F., Kaufmann, K., Ko, D.K., Kumar, S., Malkovskiy V, A., Nakayama, N., Obata, T., Otegui, M.S., Palfalvi, G., Quezada-Rodriguez, E.H., Singh, R., Uhrig, R.G., Waese, J., Van Wijk, K., Wright,R.C., Ehrhardt, D.W., Birnbaum, and K.D., Rhee, S.Y. (2021). Science Forum: Vision, challenges and opportunities for a Plant Cell Atlas. eLife *10*, e66877. DOI: 10.7554/eLife.66877
- Umeda, M., Ikeuchi, M., Ishikawa, M., Ito, T., Nishihama, R., Kyozuka, J., Torii, K.U., Satake, A., Goshima, G., and Sakakibara, H. (2021). Plant stem cell research is uncovering the secrets of longevity and persistent growth. Plant J. *106*, 326–335. DOI: 10.1111/tpj.15184

〔Review article〕

- Ishikawa, M., and Hasebe, M. (2022). Molecular mechanisms of reprogramming of differentiated cells into stem cells in the moss *Physcomitrium patens*. Curr. Opin. Plant Biol. *65*, 102123. DOI: 10.1016/j.pbi.2021.102123

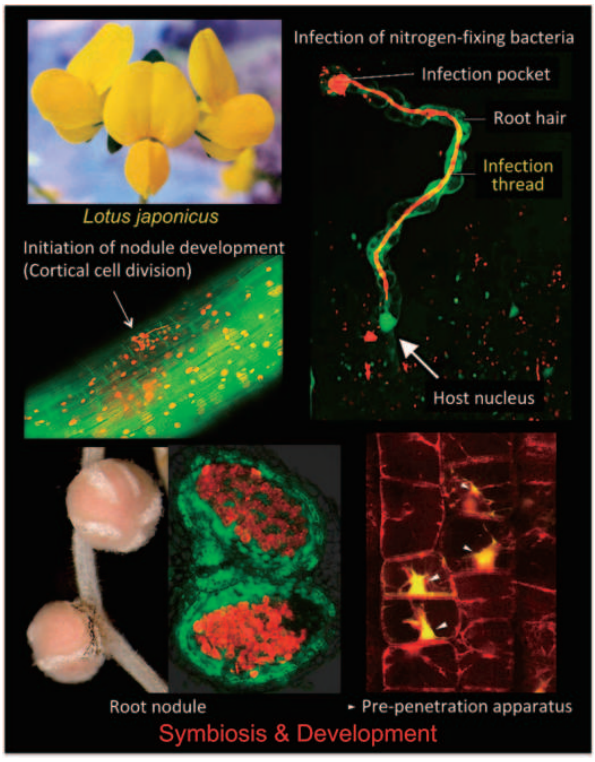
DIVISION OF SYMBIOTIC SYSTEMS

Professor
KAWAGUCHI, Masayoshi

Associate Professor
SOYANO, Takashi

Assistant Professor: *KAWADE, Kensuke*
Specially Appointed Assistant Professor: *OKUMA, Nao*
Technical Staff: *FUKADA-TANAKA, Sachiko*
Postdoctoral Fellow: *TSUGANE, Mika*, *YANO, Koji*, *HASHIMOTO, Kayo*
JSPS Postdoctoral Fellow: *FUKUDOME, Mitsutaka*
Visiting Scientist: *NAKAGAWA, Tomomi*
SOKENDAI Graduate Student: *GOTO, Takashi*
Visiting Graduate Student: *NAKAGAWA, Soya*
Technical Assistant: *TSUKAO, Chihaya*, *ODA, Akiko*
Admin Support Staff: *KOSUGI, Eiko*

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.



Visual overview of this lab's work.

I. Root nodule symbiosis

Legumes develop *de novo* organs known as root nodules to accommodate symbiotic bacteria called rhizobia. Nodule formation involves two distinct processes: rhizobial infection in epidermis and nodule development accompanied by cell division in cortex (Figure 1).

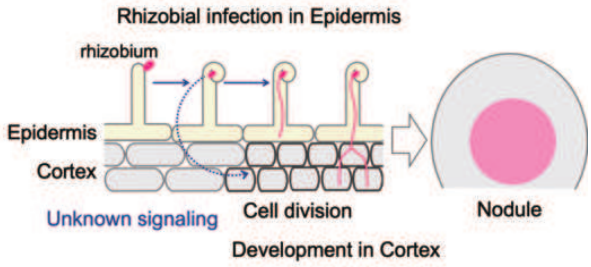


Figure 1. Processes for nodule formation.

The cortical cell division provides an indispensable scaffold for rhizobia progression from epidermis to cortex (Figure 1; pink line), leading to successful nodule formation. Cortical cell division occurs just below the site of rhizobial infection in epidermis. Therefore, there appears to be a spatiotemporal coordination across epidermis and cortex in this symbiotic organogenesis. Epidermal expression of genes required for early symbiotic genes is sufficient for nodule development in cortex, suggesting that some kinds of signals generated in epidermis trigger cortical cell division (Figure 1; blue dotted arrow). However, little is known about the mechanism that coordinates these two events.

In this study, we conducted a time-course transcriptome analysis using a *L. japonicus* non-nodulation mutant "*daphne*", which has uncoupled symbiotic events in epidermis and cortex, in that it promotes excessive rhizobial infection in epidermis but does not produce nodule primordia in cortex. Among genes that showed different expression patterns in *daphne* and wild type, we found

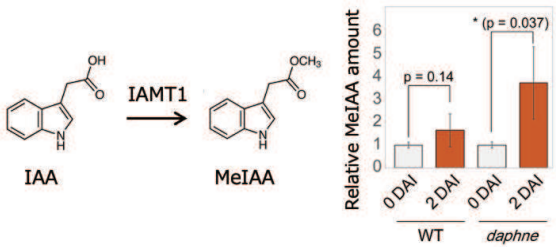


Figure 2. Auxin methylation in nodule symbiosis. IAMT1 converts IAA into MeIAA (left). Relative MeIAA amount of wild type (WT) and *daphne* at 0 (non-inoculation) or 2 d after inoculation (DAI) (right).

IAA CARBOXYL METHYLTRANSFERASE 1 (IAMT1), which encodes the enzyme that specifically converts auxin (indole-3-acetic acid; IAA) into its methyl ester (MeIAA). A significant MeIAA increase after rhizobial infection was detected by using *daphne* roots, in which excess rhizobia are infected (Figure 2).

In *Arabidopsis*, *IAMT1* reportedly serves development and differential growth of shoot. On the other hand, we found that *IAMT1* is duplicated in the legume lineage, and one of the duplicates (named *IAMT1a*) was expressed by rhizobial infection in root of *L. japonicus*. *LjIAMT1a*-knockdown inhibited nodule formation (Figure 3). *LjIAMT1a* overexpression promoted MeIAA accumulation and nodule formation. In contrast, overexpression of *LjMES17*, a MeIAA esterase, decreased MeIAA levels and nodule number. These showed that auxin methylation is essential for nodule formation.

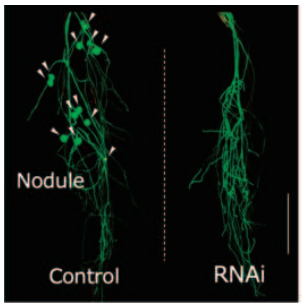


Figure 3. *LjIAMT1a*-knockdown inhibited nodule formation. Hairy roots harboring empty vector (for control; left) or *LjIAMT1a*-RNAi vector (for knock-down; right). Nodules are indicated by arrowheads. (Scale bars, 1 cm.)

Notably, application of MeIAA significantly induced expression of a symbiotic gene, *NIN*, without rhizobia (Figure 4). *NIN* is a key regulator for cortical cell division; therefore, auxin methylation could act on the process of cortical cell division. Whereas *IAMT1a* was expressed mainly in root epidermis, it would be interesting to see how MeIAA affects cortex. The result also suggests that auxin methylation is not simply due to alteration of auxin homeostasis, contrary to what many studies assumed, MeIAA, as well as other auxin secondary metabolites, is also an inactive molecule of IAA. Understanding the function of auxin methylation and MeIAA in nodule development should open a new avenue for auxin metabolisms in the plant biology.

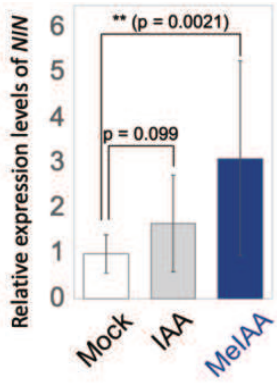


Figure 4. Exogenous MeIAA significantly induced *NIN* expression without rhizobia. Relative expression levels of *NIN* after treatment with DMSO as mock (white bar), IAA (gray bar), or MeIAA (blue bar) for 24 h.

II. Arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizal (AM) symbiosis is the oldest beneficial mutualistic relationship between the majority of terrestrial plants and AM fungi. AM fungal spores germinate and elongate their hyphae toward the root of host plants then enter the root cells and form finely branched structures, arbuscules, for exchanging nutrients with plants (Figure 5). AM fungi deliver mineral nutrients especially phosphorus in the soil to host plants. In return, they obtain organic carbon sources such as sugars which are produced through photosynthesis. AM fungi are obligate symbionts depending on their host plants for essential nutrients, thus they are incapable of propagation by themselves.

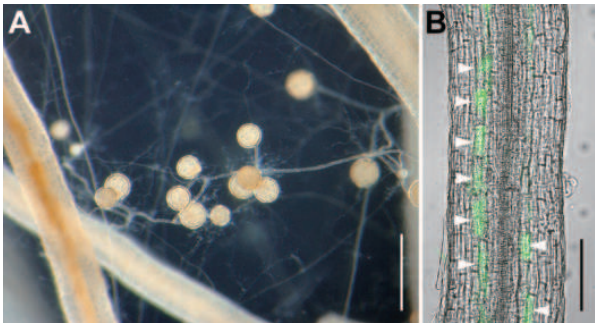


Figure 5. AM fungus *R. clarus* HR1 growing with carrot hairy roots. (A) Expanded extraradical hyphae and formed symbiotically generated spores between the roots. Bar, 500 μm. (B) Fungal structures stained by green fluorescent (Wheat germ agglutinin Oregon green 488) in the root. Arrowheads, arbuscules inside root cortical cells. Bar, 100 μm.

In the history of AM fungal culture research, it was shown that an AM fungus *Rhizophagus irregularis* could complete its life cycle in co-culture with mycorrhiza-helper bacterium *Paenibacillus validus* in the absence of host plants. Over the last several years, it was reported that AM fungi receive not only sugars but also fatty acids. Subsequently, from these experiments, it was demonstrated that some fatty acids such as palmitoleate and myristate induce *R. irregularis* spore formation under an asymbiotic (completely host-free) culture media.

We found that a fungus isolated from Aichi prefecture, *Rhizophagus clarus* HR1, whose genome has been sequenced, shows better growth on the asymbiotic culture medium, thus is more suitable for this culture method. A fatty acid, myristate promotes *R. clarus* hyphal growth, but the addition of only myristate could not ensure induction of asymbiotically-generated spores (AS) formation. Furthermore, the number of AS in this culture was much smaller than those of symbiotically-generated spores (SS) which are produced in the co-culture system with hairy roots.

Some phytohormones have been reported that they have important roles as signals during AM symbiosis. Especially, strigolactone is well known as a major signal which induces hyphal elongation and branching and stimulates their mitochondrial activity in the pre-symbiotic stage. By supplementing synthetic strigolactone, GR24 with myristic acid, the time of germination and AS formation in *R. clarus* was accelerated. Although not all *R. clarus* spores formed AS in the medium supplemented

only with myristic acid, the addition of GR24 resulted in a quite high efficiency of AS formation to nearly 100 %. Besides strigolactone, it was reported that there was an increase of jasmonate levels in AM roots and the upregulation of genes involved in jasmonic acid biosynthesis in the cells containing arbuscules. Application of jasmonates in the medium with myristic acid and GR24 led *R. clarus* to produce a higher number of AS with larger sizes (Figure 6).

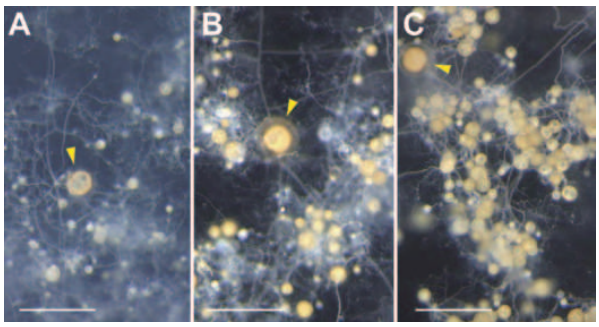


Figure 6. Produced spores on asymbiotic culture medium, supplemented with only myristate (A), myristate and GR24 (B), myristate, GR24 and methyl jasmonate (C). Arrowheads, parent spores. Bars, 500 μm.

Although the number of AS produced in the medium with myristate, GR24 and jasmonate has been close to that of SS, the size of AS is still smaller than SS. However, AS can be subcultured with the same efficiency as the first asymbiotic culture. Moreover, AS was capable of infecting hairy roots and plants. The growth of Welsh onions was significantly promoted by inoculation with AS as well as with SS (Figure 7). Our findings would lead us to more extensive studies of AM symbiosis and to the application of AS as an inoculum for basic research and for sustainable agriculture.

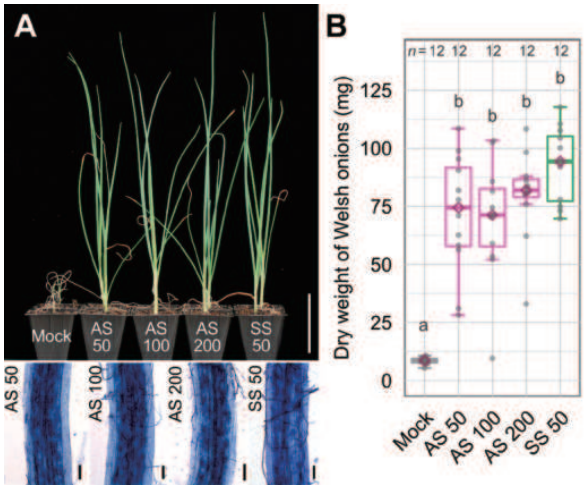


Figure 7. Inoculation of AM fungal spores to Welsh onions. Numbers following AS or SS are the number of spores for inoculation. (A) An appearance of plants inoculated with AM spores (upper). Bar, 5 cm. Ink-stained fungal structures in the roots (bottom). Bars, 50 μm. (B) Dry weight of Welsh onion shoots. *Different letters* indicate significant differences among treatments in each trial using Wilcoxon rank-sum test with Bonferroni correction, $p < 0.05$.

Publication List:

〔Original papers〕

- Goto, T., Soyano, T., Liu, M., Mori, T., and Kawaguchi, M. (2022). Auxin methylation by *IAMT1*, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus*. Proc. Natl. Acad. Sci. U.S.A. *119*, e2116549119. DOI: 10.1073/pnas.2116549119
- Hayashi-Tsugane, M., and Kawaguchi, M. (2022). *Lotus japonicus* HAR1 regulates root morphology locally and systemically under a moderate nitrate condition in the absence of rhizobia. Planta *255*, 95. DOI: 10.1007/s00425-022-03873-8
- Misawa, F., Ito, M., Nosaki, S., Nishida, H., Watanabe, M., Suzuki, T., Miura, K., Kawaguchi, M., and Suzaki, T. (2022). Nitrate transport via NRT2.1 mediates NIN-LIKE PROTEIN-dependent suppression of root nodulation in *Lotus japonicus*. Plant Cell *34*, 1844–1862. DOI: 10.1093/plcell/koac046
- Nishida, H., Nosaki, S., Suzuki, T., Ito, M., Miyakawa, T., Nomoto, M., Tada, Y., Miura, K., Tanokura, M., Kawaguchi, M., and Suzaki, T. (2021). Different DNA-binding specificities of NLP and NIN transcription factors underlie nitrate-induced control of root nodulation. Plant Cell *33*, 2340–2359. DOI: 10.1093/plcell/koab103
- Tanaka, S., Hashimoto, K., Kobayashi, Y., Yano, K., Maeda, T., Kameoka, H., Ezawa, T., Saito, K., Akiyama, K., and Kawaguchi, M. (2022). Asymbiotic mass production of the arbuscular mycorrhizal fungus *Rhizophagus clarus*. Commun. Biol. *5*, 43. DOI: 10.1038/s42003-021-02967-5
- Fukudome, M., Shimokawa, Y., Hashimoto, S., Maesako, Y., Uchi-Fukudome, N., Niihara, K., Osuki, K.I, and Uchiumi, T. (2021). Nitric oxide detoxification by *Mesorhizobium loti* affects root nodule symbiosis with *Lotus japonicus*. Microbes Environ. *36*, ME21038. DOI: 10.1264/jsme2.ME21038
- Kobayashi, Y., Katsuren, M., Hojo, M., Wada, S., Terashima, y., Kawaguchi, M., Tokuda, G., Kinjo, K., and Shigenobu, S. (2022). Taxonomic revision of *Termitomyces* species found in Ryukyu Archipelago, Japan, based on phylogenetic analyses with three loci. Mycoscience *63*, 33-38. DOI: 10.47371/mycosci.2021.11.001

〔Review articles〕

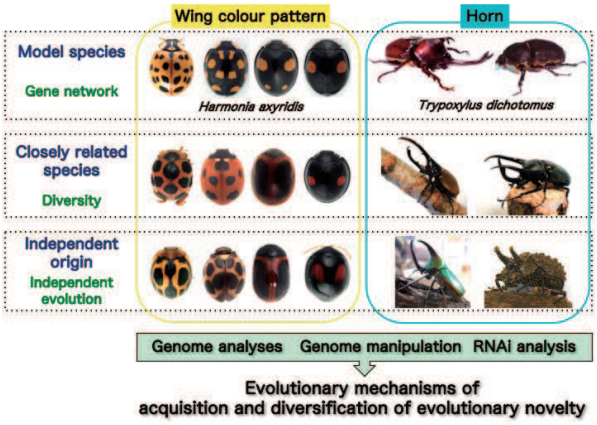
- Okuma, N., and Kawaguchi, M. (2021). Systemic optimization of legume nodulation: A shoot-derived regulator, miR2111. Front. Plant Sci. *12*, 682486. DOI: 10.3389/fpls.2021.682486
- Soyano, T., Liu, M., Kawaguchi, M., and Hayashi, M. (2021). Leguminous nodule symbiosis involves recruitment of factors contributing to lateral root development. Curr. Opin. Plant Biol. *59*, 102000. DOI: 10.1016/j.pbi.2020.102000

DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY

Professor
NIIMI, Teruyuki

Assistant Professor:	*ANDO, Toshiya*
	NAKAMURA, Taro
Specially Appointed Assistant Professor:	
	MORITA, Shinichi
Technical Staff:	*MIZUTANI, Takeshi*
Postdoctoral Fellow:	*MATSUOKA, Yuji*
	SAKURA, Kazuki
JSPS Postdoctoral Fellow:	*YANO, Koki*
Research Staff:	*CHIKAMI, Yasuhiko*
	KAWAGUCHI, Haruka
	MORITA, Junko
	TAGUCHI, Rie
SOKENDAI Graduate Student:	*KITAZAWA, Yurina*
Visiting Undergraduate:	*ODA, Hiroki*
Visiting Scientist:	*TAKENAKA, Masaki*
Technical Assistant:	*YOKOYAMA, Michiko*
	SUGIYAMA, Hiroko
Admin Support Staff:	*SAITO, Eiko*

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.



Visual overview of this lab's work.

I. Origin and diversification of insect wings

The flight organs of insects have uniquely evolved when compared to that of other various flying animals on earth. Despite more than two centuries of debate, the evolutionary origin of insect wings is still an enigma; one which we are trying to decipher 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.

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

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 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. Furthermore, 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 us understanding the origin and evolution of insect wings.

Interestingly, our previous work showed that *vg* expressing epidermal tissue forms lateral outgrowths in non-winged segments in the mealworm beetle (Ohde *et al*., 2013). Based on this, 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 for creating 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 plays various ecologically important roles such as intraspecific sexual signaling, mimesis, mimicry, and is also used 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 acts as a warning signal to predators that they taste bad. At the same time, various other insect species utilize this ecological signal by mimicking the ladybirds' wing color patterns. Mimicry provides 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 currently 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.

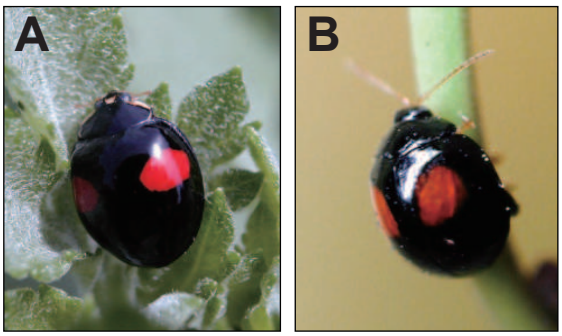


Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

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 have 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 changes 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 attempting to apply genome-editing technologies such as TALEN and CRISPR/Cas9 to tackle this issue. Thus far, we have achieved an efficient method of gene disruption using TALEN. Recently, we have begun successfully establishing the disrupting *pannier* gene function using CRISPR/Cas9 system. Ribonucleoprotein (RNP) complex of Cas9 protein and guide RNA targeting *pannier* coding exon was microinjected into the fertilized eggs. As a result, over 60% of G0 founders produced *pannier* knockout progenies (Figure 3). CRISPR/cas9 mediated gene disruption has advantages in terms of ease of designing gRNA and also possesses a high KO efficiency compared to TALEN mediated gene modification in *Harmonia*. To this end, we are continuing to develop CRISPR/Cas9 mediated gene knock-in method. To apply this approach, we are trying to attempt 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.

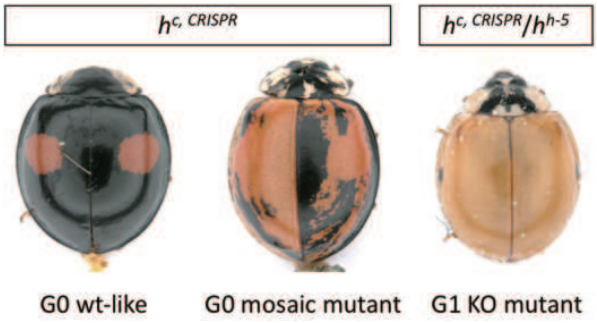


Figure 3. Representative photographs of *pannier* mutant phenotypes in G0 wt-like (left side), G0 mosaic (middle side) and G1 KO mutant (right side).

We are also establishing cryopreservation methods for germline cells in ladybird beetles to assist us with this. This is due to 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.

We plan to eventually 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 de novo multiple times 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 among the best models for studying how an extreme, sex-specific morphological structure is formed (Figure 4 A).

We first investigated the process of horn primordium formation to understand the developmental mechanism of horn formation. The insect body is covered by a rigid exoskeleton mainly consisting of chitin, so changes in body size and shapes are produced by molting. Beetle horns first appear at the pupal period (Figure 4 A). During the prepupal period (about 130 hours) which is an intermediate stage between the last larval instar stage and pupation, the head horn primordia are packed folded inside the larval head capsule (Figure 4 B). This folded horn primordium develops into its final 3D shape (pupal horn) during pupation. To properly make the horn during pupation, the folding pattern (Figure 4 B) and surface micro furrows (Figure 4 C) are precisely formed on the horn primordium and packed within the confined space of the prepupal head. We recently attempt to use micro-CT to analyze 3D pattern formation of head horn primordia during the prepupal period. We found that horn 3D morphogenesis is accomplished in only 72 hours from the prepupal initiation (Figure 4 B, C).

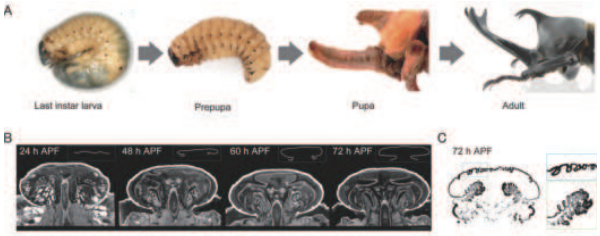


Figure 4 Morphological change of horn primordia in *Trypoxylus dichotomus*.
(A) Developmental process of *Trypoxylus dichotomus* male. (B, C) Micro-CT images of the male head horn primordium during pupal development. (Adapted from Morita and Niimi, *Sanshi Konchu Biotec*, *86*: 145–151, 2020.)

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 pursue the evolutionary origin of such a regulatory system.

Publication List:

〔Original papers〕

- Hashiro, S., Chikami, Y., Kawaguchi, H., Krylov, A.A., Niimi, T., and Yasueda, H. (2021). Efficient production of long double-stranded RNAs applicable to agricultural pest control by *Corynebacterium glutamicum* equipped with coliphage T7-expression system. Appl. Microbiol. Biotechnol. *105*, 4987–5000. DOI: 10.1007/s00253-021-11324-9
- Miyazaki, S., Fujiwara, K., Kai, K., Masuoka, Y., Gotoh, H., Niimi, T., Hayashi, Y., Shigenobu, S., and Maekawa, K. (2021). Evolutionary transition of *doublesex* regulation from sex-specific splicing to male-specific transcription in termites. Sci. Rep. *11*, 15992. DOI: 10.1038/s41598-021-95423-7
- Ohde, T., Mito, T., and Niimi, T. (2022). A hemimetabolous wing development suggests the wing origin from lateral tergum of a wingless ancestor. Nat. Commun. *13*, 979. DOI: 10.1038/s41467-022-28624-x
- Ylla, G., Nakamura, T., Itoh, T., Kajitani, R., Toyoda, A., Tomonari, S., Bando, T., Ishimaru, Y., Watanabe, T., Fuketa, M., Matsuoka, Y., Barnett, A.A., Noji, S., Mito, T., and Extavour, C.G. (2021). Insights into the genomic evolution of insects from cricket genomes. Commun. Biol. *4*, 733. DOI: 10.1038/s42003-021-02197-9

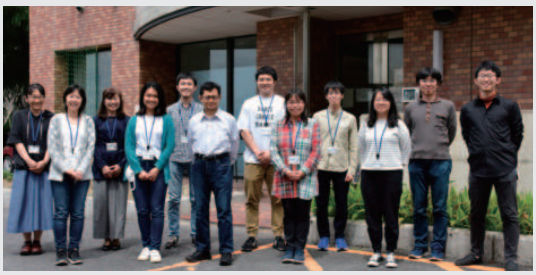
〔Review article〕

- Niimi, T., and Ando, T. (2021). Evo-devo of wing colour patterns in beetles. Curr. Opin. Genet. & Dev. *69*, 97–102. DOI: 10.1016/j.gde.2021.02.007

Laboratory of Evolutionary Genomics

Professor:
SHIGENOBU, Shuji

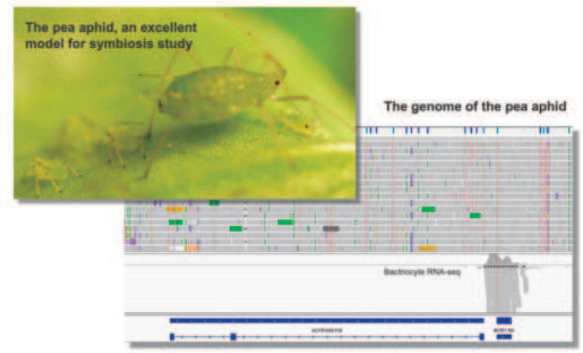
Specially Appointed Assistant Professor: *YODA, Shinichi*
Postdoctoral Fellow: *KOBAYASHI, Yuuki*
JSPS Postdoctoral Fellow: *NOZAKI, Tomonari*
MATSUDA, Naoki
SOKENDAI Graduate Student: *YORIMOTO, Shunta*
TAN, Kathrine
Visiting Scientist : *HUANGH, Jia-Hsin*
TONG, Xin
CHUNG, Chen-yo
Technical Assistant: *SUZUKI, Miyuzu*
WEN, Hsin-I
IKEDA, Mika
Admin Support Staff: *ICHIKAWA, Mariko*



Overview

"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.



Visual overview of this lab's work.

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, 2010; Shigenobu *et al*., 2000; Shigenobu & Yorimoto, 2022). 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, 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*., 2019). 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.

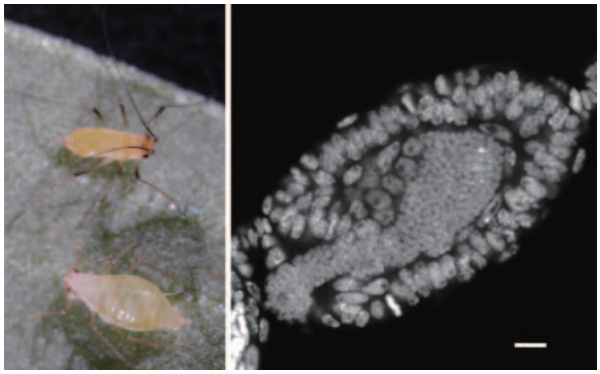


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20 um.

Genomics of emerging model insects

Recent remarkable technological advancements allow us to study any organisms at molecular and genetic levels, which has been difficult for non-model organisms. Insect science is also benefited by the technological innovation. New entomology is starting to study the amazing variety of morphology, phisiology and ethology of insects by using these state-of-the-art technologies. Our group is developing a platform for emerging model insects to read the genomes/transcriptomes and then manipulate their genomes.

Termites are emerging model social organisms characterized by a sophisticated caste system. We recently sequenced the genome of the subterranean termite *Reticulitermes sepratus* (Shigenobu *et al*., 2022). The analyses of ~1.0 Gb genome and 15,591 genes revealed the significance of gene duplication in social evolution. Gene duplication associated with caste-biased gene expression was prevalent in the termite genome. The duplicate genes comprised diverse categories related to social functions, such as chemical communication, social immunity and defense, and they were often expressed in a caste-specific organ. Gene duplication may facilitates social evolution through regulatory diversification leading to caste-biased expression and functional specialization.

Beyond insect genomics, we are involved in several genome projects of emerging model organisms. We recently published the genome information of a photosynthetic sea slug. Some sea slugs take up chloroplasts from the algae that they consume into their cells. These chloroplasts retain their ability to perform photosynthetic activity within the animal cells for several months, and thus provide them with photosynthesis-derived nutrition. This process is called kleptoplasty. Our genome analysis of *Plakobranchus ocellatus* revealed the chloroplast acquisition without gene transfers in the photosynthetic sea slug (Maeda *et al*., 2021).

Caste development and symbiosis in the social aphid *Ceratovacuna japonica*

Eusociality has evolved repeatedly among insect lineages and represents one of the major transitions in evolution. Molecular mechanisms underlying sociality have been extensively studied with hymenopteran lineages, such as ants, bees, and wasps, and termites. Some aphid species show eusociality and important groups for better understanding of the eusocial evolution, since they are one of the primitive eusocial species that belong to Hemiptera. We focus on the social aphid *Ceratovacuna japonica*, because we recently established the laboratory culture system. First, we investigated the sterility regulation in the soldier caste of the aphids (Chung and Shigenobu, 2022). We found that soldiers, that are completely sterile, possess a pair of ovarioles, but soldiers' ovarioles were small and lacking gastrulating embryos. We also found that soldier reproduction is constrained by apoptosis in maternal nutritive cells and necrosis in oocytes and embryos. *C. japonica* also contains endosymbionts. The eusocial aphid *C. japonica* provides us with a unique opportunity to study how symbiosis operates in a social setting. We are currently investigating the interface of symbiosis and sociality.

Publication List

〔Original papers〕

- Igawa-Ueda, K., Ikuta, T., Tame, A., Yamaguchi, K., Shigenobu, S., Hongo, Y., Takaki, Y., Fujikura, K., Maruyama, T., and Yoshida, T. (2021). Symbiont Transmission onto the Cell Surface of Early Oocytes in the Deep-sea Clam *Phreagena okutanii*. Zoolog. Sci. *38*, 140–147. DOI: 10.2108/zs200129
- Kobayashi, Y., Katsuren, M., Hojo, M., Wada, S., Terashima, Y., Kawaguchi, M., Tokuda, G., Kinjo, K., and Shigenobu, S. (2022). Taxonomic revision of *Termitomyces* species found in Ryukyu Archipelago, Japan, based on phylogenetic analyses with three loci. Mycoscience *63*, 33–38. DOI: 10.47371/mycosci.2021.11.001
- Korgaonkar, A., Han, C., Lemire, A.L., Siwanowicz, I., Bennouna, D., Kopec, R.E., Andolfatto, P., Shigenobu, S., and Stern, D.L. (2021). A novel family of secreted insect proteins linked to plant gall development. Curr. Biol. *31*, 1836-1849.e12. DOI: 10.1016/j.cub.2021.01.104
- Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., and Shigenobu, S. (2021). Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*. eLife *10*, e60176. DOI: 10.7554/eLife.60176
- Miyazaki, S., Fujiwara, K., Kai, K., Masuoka, Y., Gotoh, H., Niimi, T., Hayashi, Y., Shigenobu, S., and Maekawa, K. (2021). Evolutionary transition of doublesex regulation from sex-specific splicing to male-specific transcription in termites. Sci. Rep. *11*, 15992. DOI: 10.1038/s41598-021-95423-7
- Nozaki, T., Suetsugu, K., Sato, K., Sato, R., Takagi, T., Funaki, S., Ito, K., Kurita, K., Isagi, Y., and Kaneko, S. (2021). Development of microsatellite markers for the geographically parthenogenetic stick insect *Phraortes elongatus* (Insecta: Phasmatodea). Genes Genet. Syst. *96*, 199–203. DOI: 10.1266/ggs.21-00022
- Nozaki, T., and Matsuura, K. (2021). Oocyte resorption in termite queens: Seasonal dynamics and controlling factors. J. Insect Physiol. *131*, 104242. DOI: 10.1016/j.jinsphys.2021.104242
- Nozaki, T., Hojo, M., Yashiro, T., and Matsuura, K. (2021). First Record of the Soil-feeding Higher Termites (Isoptera: Termitidae) from Okinawa Island, the Central Ryukyus, Japan. SOCIOBIOLOGY *68*, e6159. DOI: 10.13102/sociobiology.v68i2.6159
- Shigenobu, S., Hayashi, Y., Watanabe, D., Tokuda, G., Hojo, M.Y., Toga, K., Saiki, R., Yaguchi, H., Masuoka, Y., Suzuki, R., Suzuki, S., Kimura, M., Matsunami, M., Sugime, Y., Oguchi, K., Niimi, T., Gotoh, H., Hojo, M.K., Miyazaki, S., Toyoda, A., Miura, T., and Maekawa, K. (2022). Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: Gene duplication facilitates social evolution. Proc. Natl. Acad. Sci. U.S.A. *119*, e2110361119. DOI: 10.1073/pnas.2110361119
- Takeuchi, T., Matsubara, H., Minamitani, F., Satoh, Y., Tozawa, S., Moriyama, T., Maruyama, K., Suzuki, K.T., Shigenobu, S., Inoue, T., Tamura, K., Agata, K., and Hayashi, T. (2022). Newt Hoxa13 has an essential and predominant role in digit formation during development and regeneration. Development *149*, dev200282. DOI: 10.1242/dev.200282
- Tanaka, S., Hashimoto, K., Kobayashi, Y., Yano, K., Maeda, T., Kameoka, H., Ezawa, T., Saito, K., Akiyama, K., and Kawaguchi, M. (2022). Asymbiotic mass production of the arbuscular mycorrhizal fungus Rhizophagus clarus. Commun. Biol. *5*, 43. DOI: 10.1038/s42003-021-02967-5
- Tominaga, T., Miura, C., Sumigawa, Y., Hirose, Y., Yamaguchi, K., Shigenobu, S., Mine, A., and Kaminaka, H. (2021). Conservation and Diversity in Gibberellin-Mediated Transcriptional Responses Among Host Plants Forming Distinct Arbuscular Mycorrhizal Morphotypes. Front. Plant Sci. *12*, 795695. DOI: 10.3389/fpls.2021.795695
- Tong, X., Kanzaki, N., and Akimoto, S. (2021). First record of a mermithid nematode (Nematoda: Mermithidae) parasitizing winged females of gall-forming aphids (Hemiptera: Aphididae: Eriosomatinae). Entomol. Sci. *24*, 354–360. DOI: 10.1111/ens.12486
- Yashiro, T., Tea, Y.-K., van der Wal, C., Nozaki, T., Mizumoto, N., Hellemans, S., Matsuura, K., and Lo, N. (2021). Enhanced

heterozygosity from male meiotic chromosome chains is superseded by hybrid female asexuality in termites. Proc. Natl. Acad. Sci. U.S.A. *118*, e2009533118. DOI: 10.1073/pnas.2009533118

- Yoshida, Y., Shaikhutdinov, N., Kozlova, O., Itoh, M., Tagami, M., Murata, M., Nishiyori-Sueki, H., Kojima-Ishiyama, M., Noma, S., Cherkasov, A., Gazizova, G., Nasibullina, A., Deviatiiarov, R., Shagimardanova, E., Ryabova, A., Yamaguchi, K., Bino, T., Shigenobu, S., Tokumoto, S., Miyata, Y., Cornette, R., Yamada, T.G., Funahashi, A., Tomita, M., Gusev, O., and Kikawada, T. (2022). High quality genome assembly of the anhydrobiotic midge provides insights on a single chromosome-based emergence of extreme desiccation tolerance. NAR Genomics Bioinforma. *4*, lqac029. DOI: 10.1093/nargab/lqac029

〔**Review articles**〕

- Shigenobu, S. & Yorimoto, S. Aphid hologenomics: current status and future challenges. Curr Opin Insect Sci*4*, 100882. DOI:10.1016/j.cois.2022.100882.
- Shigenobu, S. (2022). Editorial overview: Insect genomics: Genomics of emerging model insects. Curr. Opin. Insect Sci. *51*, 100913. DOI: 10.1016/j.cois.2022.100913
- Miura, T., Oguchi, K., Yamaguchi, H., Nakamura, M., Sato, D., Kobayashi, K., Kutsukake, N., Miura, K., Hayashi, Y., Hojo, M., Maekawa, K., Shigenobu, S., Kano, T., and Ishiguro, A. (2022). Understanding of superorganisms: collective behavior, differentiation and social organization. Artif. Life Robot. *27*, 204–212. DOI: 10.1007/s10015-022-00754-x

LABORATORY OF BIORESOURCES

Specially Appointed Professor
NARUSE, Kiyoshi

Research Staff:	*KANEKO, Hiroyo*
	HARA, Ikuyo
	YANOGAWA, Azusa
Visiting Scientist:	*SATO, Tadashi*
	NITTA, Masato
Visiting Graduate Student:	*IMAEDA YASSUMOTO, Tamiris*
Technical Assistant:	*AJIOKA, Rie*
	KOIKE, Chieko
	KOIKE, Yukari
	TESHIMA, Yuuko
	TORII, Naoko
	YAMAZAKI, Toko
Admin Support Staff:	*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 accsssibility 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.

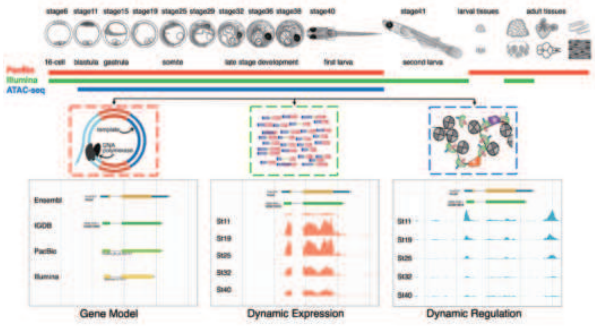


Figure 1. Overview of medaka multi-omics anslysis during development

II. The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel and their genomic variation and epigenomic landscape

The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel is the first near-isogenic panel of 80 inbred lines derived from a wild founder population collected from Kiyosu, Toyohashi. Inbred lines provide fixed genomes that make it possible to repeat studies, to vary both genetic information and environmental conditions in controlled studies, as well as to conduct functional studies. MIKK will thus make it possible to investigate phenotype-to-genotype association studies of complex genetic traits while carefully controlling interacting factors. Its applications are numerous, and include genetic research, human health, drug development, and fundamental biology. Using short reads from Illumina, we further characterise each of the 80 MIKK lines according to their genetic composition, using the Southern Japanese medaka inbred strain HdrR as a reference genome. We used Oxford Nanopore long read sequencing technology to analyze 12 representative lines from MIKK to analyze the larger and more complex structural variations. We also investigate line-specific CpG methylation and performed differential DNA methylation analysis across these 12 lines.

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. National BioResource Project Medaka

Our laboratory has been acting as main facility of the Medaka BioResource Project (NBRP Medaka) in the fourth phase of NBRP, which started in 2017. We are providing wild strains, and related species, genomic resources (approximately 400,000 cDNA clones from 30 different cDNA libraries (containing approximately 23,000 different sequences) and BAC/Fosmid clones covering the entire medaka genome), and hatchery enzymes essential for embryo manipulation and live imaging. These bioresources are available on our website (Figure 3. https://shigen.nig.ac.jp/medaka/). These bioresources can be searched by various methods such as keywords, sequence homology, and expression profiles using the database on the website. In addition, genome browsers for the three inbred strains, phylogenetic relationships between wild strains of medaka and related species, and experiment manuals are also available. With the approval of the second supplementary budget for FY2020, we were able to install a cabinet-type fish tank washing machine. This has freed up our technical support staff from the need to wash the tanks by hand, and has allowed us to focus more on breeding and management, which requires more human work. In addition, a system to remotely monitor the temperature, humidity, and illumination in the medaka breeding rooms and the water temperature in the breeding tanks was installed. The air conditioner in the breeding room was also upgraded. We have continuously monitored the medaka breeding conditions using these systems.

Figure 2. NBRP Medaka website

Publication List:

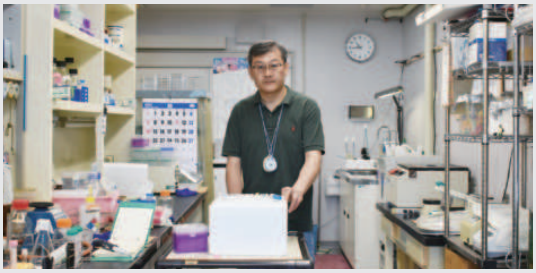
〔Original papers〕

- Fitzgerald, T., Brettell, I., Leger, A., Wolf, N., Kusminski, N., Monahan, J., Barton, C., Herder, C., Aadepu, N., Gierten, J., Becker, C., Hammouda, O.T., Hasel, E., Lischik, C., Lust, K., Sokolova, N., Suzuki, R., Tsingos, E., Tavhelidse, T., Thumberger, T., Watson, P., Welz, B., Khouja, N., Naruse, K., Birney, E., Wittbrodt, J., and Loosli, F. (2022). The Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel. Genome Biol. *23*, 59. DOI: 10.1186/s13059-022-02623-z
- Leger, A., Brettell, I., Monahan, J., Barton, C., Wolf, N., Kusminski, N., Herder, C., Aadepu, N., Becker, C., Gierten, J., Hammouda, O.T., Hasel, E., Lischik, C., Lust, K., Sokolova, N., Suzuki, R., Tavhelidse, T., Thumberger, T., Tsingos, E., Watson, P., Welz, B., Naruse, K., Loosli, F., Wittbrodt, J., Birney, E., and Fitzgerald, T. (2022). Genomic variations and epigenomic landscape of the Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel. Genome Biol. *23*, 58. DOI: 10.1186/s13059-022-02602-4
- Seleit, A., Ansai, S., Yamahira, K., Masengi, K. W., Naruse, K., & Centanin, L. (2021). Diversity of lateral line patterns and neuromast numbers in the genus *Oryzias*. Journal of Experimental Biology, *224*, jeb242490. DOI: 10.1242/jeb.242490

LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA, Yoshiaki*



Tor (target of rapamycin) is a Ser/Thr protein kinase which is well conserved in organisms spanning from yeasts to mammals. 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 (especially the translation step), the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. Up until now, it's been deemed unlikely that TORC2 can recognize nutrient signals.

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

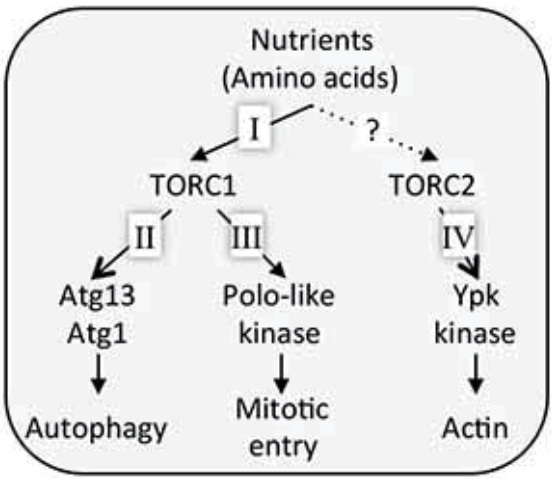


Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

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

We have conducted genetic research and discovered the involvement of (aminoacyl-) tRNA in TORC1 regulation. For example, mutants of aminoacyl-tRNA synthetases (ARSs) exhibited inactivation of TORC1 even under amino acid-rich condition, suggesting that aminoacyl-tRNA, a product of ARS acts as an amino acid signal rather than an amino acid itself. Biochemical *in vitro* TORC1 assay also revealed that tRNA directly inhibits TORC1 kinase activity, suggesting that uncharged tRNA, produced under amino acid-depleted condition, functions as a starvation signal. Reducing cellular tRNA molecules desensitizes TORC1 inactivation by nitrogen starvation *in vivo*.

Based on these results, a TORC1 regulatory model was proposed which 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).

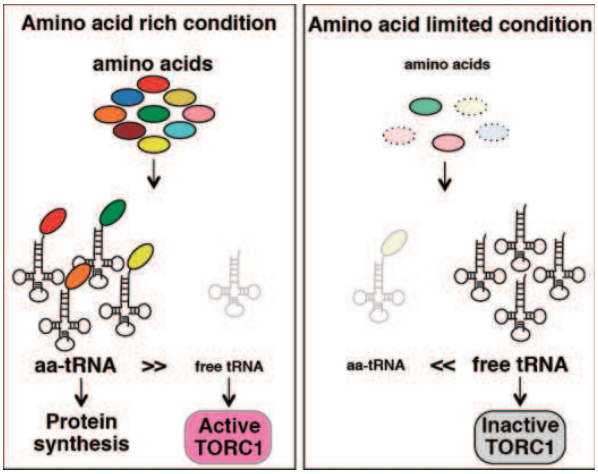


Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

Since tRNA directly inhibits TORC1 activity, TORC1 should have a tRNA-binding site(s). Thus, we have investigated further so as to determine the tRNA-binding site. So far, we have obtained a good candidate for the tRNA-binding site in Tor protein, and we will now focus on this domain to determine its function.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

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

We have been 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 consequent induction of autophagy.

III. Relationships between TORC1 and eIF4E-binding proteins

EIF4E-binding proteins (4EBPs), which binds to translation initiation factor 4E (eIF4E), are thought to negatively regulate translation initiation, because their competition infused binding to eIF4E prevents eIF4E-eIF4G association, which is a primary and essential procedure in translation initiation. In mammals, the inhibitory function of 4EBP1 is regulated by mammalian TORC1 (mTORC1). Under nutrient-rich conditions, mTORC1 phosphorylates 4EBP1, and the phosphorylated 4EBP1, lose affinity to eIF4E and let eIF4E bind to eIF4G.

We examined whether two yeast 4EBPs, Caf20 and Eap1, have properties in common with 4EBP1. Caf20, but not Eap1, is phosphorylated in a TORC1-dependent manner, it binds to eIF4E, and it never associates to eIF4G. However, Caf20 was not directly phosphorylated by TORC1, Caf20-eIF4E binding was not affected by TORC1 activity, and Caf20-eIF4E complex was found in ribosome fraction, suggesting that the function of the yeast 4EBP is different from that of its mammalian counterparts.

Publication List:

〔**Original paper**〕

● Sekiguchi, T., Ishii, T., Kamada, Y., Funakoshi, M., Kobayashi, H., and Furuno, N. (2022). Involvement of Gtr1p in the oxidative stress response in yeast *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. *598*, 107–112. DOI: 10.1016/j.bbrc.2022.02.016

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

Assistant Professor: *HOSHINO, Atsushi*
Technical Assistant: *TAKEUCHI, Tomoyo*
ITO, Kazuyo
KAWADA, Saki

Genomic structures and their genetic information are stably transmitted into daughter cells and future generations during cell division; however, they can vary genetically and/or epigenetically. Such variability impacts gene expression and evolution. To understand these genomic dynamics in eukaryotes, particularly 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 a uniformly pigmented corolla. For our study, we collected mutants displaying pigmentation patterns. Flower pigmentation patterns are easily observable and the molecular mechanisms underlying these phenomena provide useful model systems for investigating genomic variability.

Recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers caused by a stable insertion of a transposable element into a gene for flower pigmentation, regulated by epigenetic mechanisms (Figure 1). We intend to analyze detailed molecular mechanisms of these mutations.

II. Whole-genome analysis of various *I. nil* lines

The National BioResource Project (NBRP) Morning Glory (described below) maintains ~3,500 lines, including 2 standard lines: the Tokyo Kokei Standard and Violet. A high-quality draft whole-genome sequence of the Tokyo Kokei Standard, which is accessible from our database, has been published. The NBRP Genome Information Upgrading Program supported whole-genome sequencing of 100 representative lines. We chose lines based on the needs of the research community and to provide information regarding their polymorphisms and gene mutations. We included the multiple mutants called "Henka-Asagao," which covered the major mutations, and the wildtype plants isolated from natural populations outside Japan. From this, we discovered a total of 25,000 *Tpn1* transposon-induced insertion polymorphisms and SNPs, Indels, and CNVs at 25 million loci. Several of those polymorphisms were tightly linked to morphological mutations, including the mutations that "Henka-Asagao" carry.

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

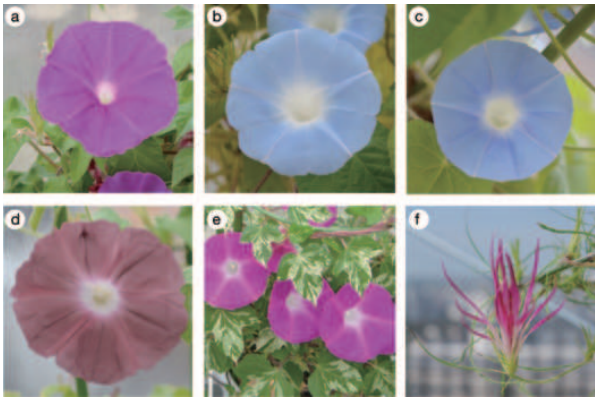


Figure 2. The *I. nil* lines used for the whole-genome analysis. (a) Violet: The most widely used standard line. (b) Africa: The wildtype line isolated in Guinea in 1956. (c) Nepal: The wildtype line isolated by Dr. Sasuke Nakao in Nepal in 1952. (d) Danjuro, one of the most popular cultivars, named after the reddish-brown color of the costume used by Danjuro Ichikawa II in his Kabuki play "Shibaraku." (e) The *tiny* mutant has small organs. White spots on the leaves are due to the *variegated* mutation. (f) A mutant line of "Henka-Asagao" showing double flowers with narrow flower petals and sepals.

III. Flower opening and time control

The time of flower opening is important for the reproductive strategy of plants, and many plants are known to flower at specific times during the day. However, little is known about the molecular mechanisms that determine flower opening time as well as flower opening itself. As their name suggests, morning glories typically bloom in the early morning. There is significant physiological knowledge about the circadian rhythm that controls flower opening time. To elucidate these molecular mechanisms, we have begun a functional analysis of genes for petal opening and the circadian rhythm.

IV. Morning glory bio-resources

NIBB is the subcenter for the NBRP dedicated to morning glories. In this project, we collect, maintain, and distribute 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 related genetic studies. Our collection includes 241 lines and 177,000 DNA clones. The whole-genome sequence, the transcriptome

sequences, and 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:

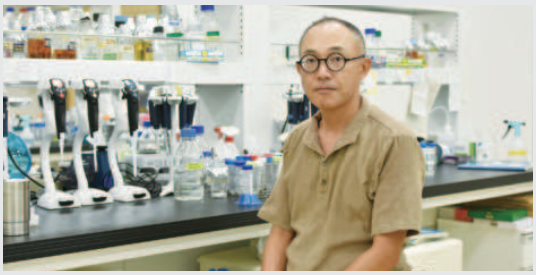
〔Original paper〕

● Nagaki, K., Furuta, T., Yamaji, N., Kuniyoshi, D., Ishihara, M., Kishima, Y., Murata, M., Hoshino, A., and Takatsuka, H. (2021). Effectiveness of Create ML in microscopy image classifications: a simple and inexpensive deep learning pipeline for non-data scientists. Chromosome Res. *29*, 361–371. DOI: 10.1007/s10577-021-09676-z

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 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 (*Oryza sativa* subsp. *japonica*), and Basmati (*Oryza sativa* subsp. *indica*). Various mutations caused by the insertion of nDart have been screened for characteristic phenotypes.

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 Koshihikari-nDart tagging line bore slightly larger grains (Figure 1) as a dominant inheritance. Transposon-display identified the insertion site of *nDart1* in the *Lgg* mutant.

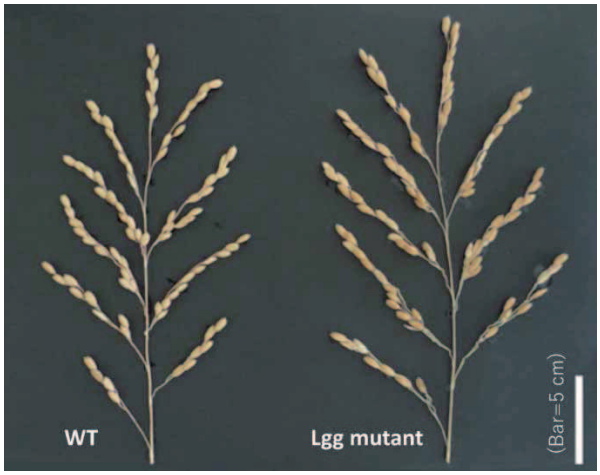


Figure 1. Phenotype of Large gain (*Lgg*). Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

The identified *LGG* gene shows similarity to RNA binding proteins. To investigate the subcellular localization of LGG protein, green fluorescent protein (GFP)-fused constructs driven by 35S promoter, 35S:LGGNP-GFP was transformed into rice calli. GFP fluorescence spots were observed in the nuclei in calli. These results suggest that LGG is localized to the nucleus. To verify that LGG was responsible for the long grain phenotype, knockout (GE) and overexpression (OE) lines were produced by transforming a CRISPR/Cas9 construct targeting the RNA recognition motif region of LGG and an LGG construct with its WT promoter into into cv. Nipponbare (NP), respectively (Figure 2(A)). Several independent GE lines showed significantly increased grain length, and three independent OE lines had shorter grains than NP. Any significant differences of panicle length, number of panicles per plant and number of spikelets per panicle were not observed among NP, GE and OE except for culm length. Observations of longitudinal sections from lemmas of GE and OE lines revealed that the cell sizes of GE and OE lines were comparable to NP (Figure2 B-C), suggesting that LGG might regulate the longitudinal cell number of spikelet hull and thus grain length.

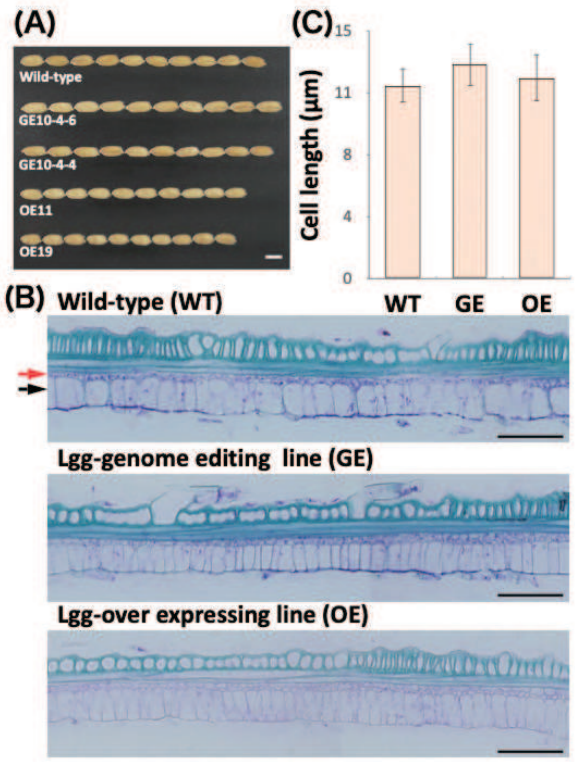


Figure 2. The spikelets morphology and longitudinal sections and cell length of the lemma of NP, GE, and OE, respectively. (A) The spikelets. Bar = 5 mm. (B) The Section of lemma in transgenic plants. The red and black arrows indicate adaxial epidermis and chlorophyll-contained parenchyma cell. (C) Cell length of lemma in NP, GE and OE. n = 3. mean ± SD.

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

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

Computer programs that accurately capture and measure the features from images that express the phenomenon become an inevitable tool to objectively describe and evaluate a biological phenomenon obtained as digital imaging data. From this point of view, we are developing image analysis programs for each experimental case and analyzing image data that captures various biological phenomena.

I. Cell segmentation/tracking system

To better understand organ formation mechanisms, it is necessary to analyze individual cells' morphology and dynamics quantitatively. However, it is difficult to achieve it due to the large image volume size generated by such as time-lapse microscopy and its ambiguity.

To unveil organogenesis from the point of view of distinct cell behaviors, we are developing application software capable of describing cell dynamics from time-lapse imaging data sets by employing image processing techniques. To observe this, we are developing a software pipeline which will automatically recognize individual cell shapes out of 3D space and tracks them through time. This system extracts cell boundaries and reconstitutes cell shapes in the form of 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 at a single-cell resolution (Figure 1). These morphometric quantities allow us to perform comparative studies on shapes and behaviors more precisely under several experimental conditions to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system.

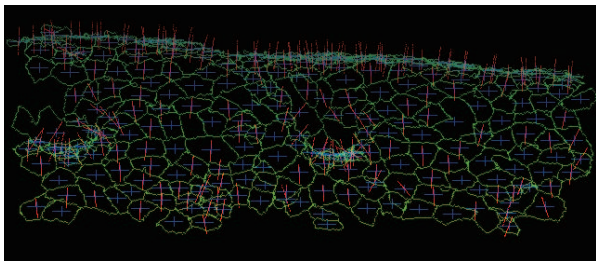


Figure 1. Visualized apical cell surface of Drosophila embryonic epidermal cells. A time-lapse confocal microscopic data set expressing E-cadherin-GFP was processed. Cell boundaries (green), the center of gravity (blue), and normal vector (magenta) are indicated for each cell.

II. Image processing pipeline for morphogenesis

In complex organs, methods for identifying and tracking the initial positions of the cell groups consisting of the final organ morphology are essential analytical methods for elucidating the developmental program.

Here, we analyzed the details of the pathways of individual cells in a brain primordium throughout the brain morphogenesis in chick embryos in its early stages. We identified the cell positions out of randomly fluorescently labeled brain primordium cells from a series of time-lapse images and constructed a database of their time evolution. Based on this database, we developed a software system for automating the visualization and quantification of the local migration pattern of brain primordium constituent cells from several experimental conditions.

III. Quantification of fish body shape

Techniques for quantitatively analyzing the movements and body forms of model organisms are considered indispensable for phenotypic evaluation in the process of elucidating the molecular mechanisms underlying the behavior of living animals.

To elucidate the mechanism of neuronal development of zebrafish, we have developed a system that extracts the body region from each frame of a video of free migration of fish juveniles taken at a high frame rate and describes the morphology from the head to the tail as a free curve model. From these curve models, we developed a system that classifies and simplifies the continuous bending forms of fish as a small number of classes. This system was applied to the movies acquired under various experimental conditions and successfully described and statistical evaluation of the phenotype.

Publication List:

〔Original paper〕

- Yoshihi, K., Kato, K., Iida, H., Teramoto, M., Kawamura, A., Watanabe, Y., Nunome, M., Nakano, M., Matsuda, Y., Sato, Y., Mizuno, H., Iwasato, T., Ishii, Y., and Kondoh, H. (2022). Live imaging of avian epiblast and anterior mesendoderm grafting reveals the complexity of cell dynamics during early brain development. Development *149*, dev199999. DOI: 10.1242/dev.199999
- Kawano, K., Kato, K., Sugioka, T., Kimura, Y., Tanimoto, M., and Higashijima, S. (2022). Long descending commissural V0v neurons ensure coordinated swimming movements along the body axis in larval zebrafish. Sci. Rep. *12*, 4348. DOI: 10.1038/s41598-022-08283-0

LABORATORY OF BIOLOGICAL DIVERSITY

OHTA Group

Specially Appointed Assistant Professor:
OHTA, Yusaku

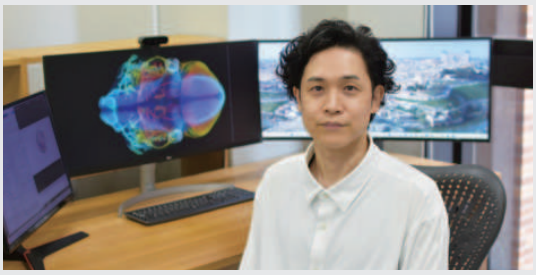


Image analysis is an important component of understanding life sciences. It enables us to quantify phenomena by extracting meaningful information from a large number of images and to properly represent that information. I am developing image analysis techniques aimed at analyzing the developmental process of early zebrafish embryo at whole embryo scale and single-cell resolution. Currently, I am developing an analysis technique that can simultaneously extract multiple pieces of information such as cell morphology, cell motility, and cell dynamics by combining 3D cell tracking and functional imaging.

I. Simultaneous multifunctional analysis of early embryonic development at a whole embryo scale and single cell resolution.

Three-dimensional remodeling of cell populations through cell migration is essential in early embryogenesis. Cell migration is highly coordinated by controlling cell-cell adhesion. To elucidate the principles of such complex embryogenesis, it is necessary to understand the cellular dynamics of the whole embryo at single cell resolution. I am developing image analysis techniques to analyze the developmental process of early zebrafish embryogenesis at whole-embryo scale and single-cell resolution.

With the development of microscopy, imaging of early embryonic imaging has evolved from two-dimensional, embryonic development has evolved from two-dimensional, fixed specimen and partial embryo observation to three-dimensional, living specimen and whole embryo scale. This evolution has led to an explosion in the analysis of cell migration during the development of early embryos in recent years. However, conventional image analysis techniques could only extract information on cell migration in early development. Therefore, I have developed an analysis method that can simultaneously extract information on cell migration, cell dynamics, and cell morphology at whole embryo scale and single cell resolution.

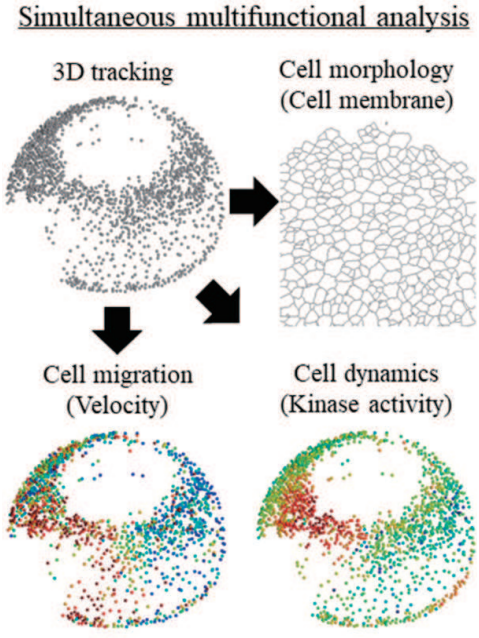


Figure 1. Simultaneous multifunctional analysis of early embryonic development

II. Research support by image analysis

The development of imaging technology in life science research has been remarkable, allowing many researchers to easily acquire large and complex image data sets. However, image analysis is still a hurdle for researchers and can become a research bottleneck. To solve this problem, I provide research support based on the following three concepts.

The first concept is quantitative image analysis based on extensive knowledge of image processing and statistics. For most researchers, image evaluation is limited to qualitative and subjective analysis. Correct analysis based on knowledge of image and statistics supports quantitative and objective analysis. The second concept is to support image analysis through machine learning, including deep learning. Machine learning has made remarkable progress in recent years, and with a little learning, it is possible to simplify analysis, which has been difficult with conventional image analysis techniques. The third concept relates to providing researchers with easy-to-understand explanations of image analysis techniques on the Web. The content ranges from the principles of image analysis methods to how to use image analysis software and plug-ins.

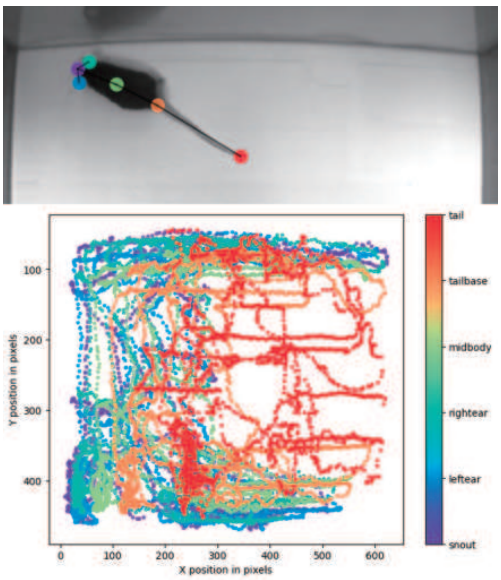
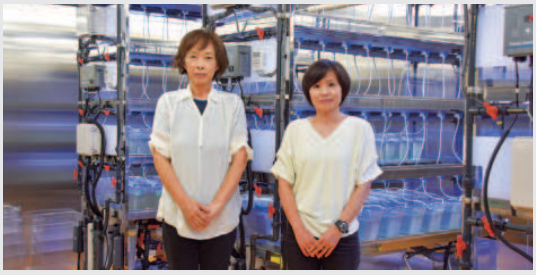


Figure 2. A website that explains how to use image analysis software

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.

A medaka habitat in summer (Miyazaki, Miyazaki Prefecture) (left). A school of wild medaka (Toyohashi, Aichi Prefecture) (right).

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.

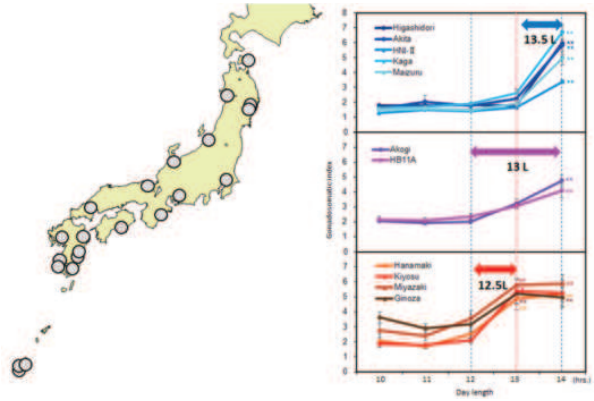


Figure 1. Differences in critical day length (right) were detected among medaka populations that had originated in various latitudes (left) .

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

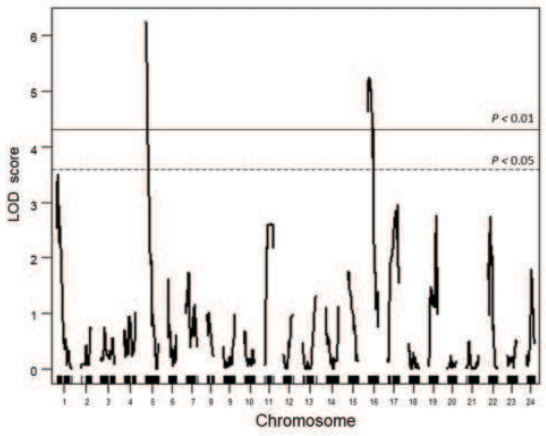


Figure 2. Result of QTL analysis for critical day length.

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 (day length, solar radiation, water temperature) 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**〕

- Nakayama, T., Okimura, K., Shen, J., Guh, Y.-J., Tamai, T.K., Shimada, A., Minou, S., Okushi, Y., Shimmura, T., Furukawa, Y., Kadofusa, N., Sato, A., Nishimura, T., Tanaka, M., Nakayama, K., Shiina, N., Yamamoto, N., Loudon, A.S., Nishiwaki-Ohkawa, T., Shinomiya, A., Nabeshima, T., Nakane, Y., and Yoshimura, T. (2020). Seasonal changes in NRF2 antioxidant pathway regulates winter depression-like behavior. Proc. Natl. Acad. Sci. U.S.A. *117*, 9594–9603. DOI: 10.1073/pnas.2000278117
- Yassumoto I, T., Nakatsukasa, M., Nagano, A.J., Yasugi, M., Yoshimura, T., and Shinomiya, A. (2020). Genetic analysis of body weight in wild populations of medaka fish from different latitudes. PLoS One *15*, *e0234803*. DOI: 10.1371/journal.pone.0234803

LABORATORY OF BIOLOGICAL DIVERSITY

SAITO Group

Specially Appointed Associate Professor
SAITO, Nen

Postdoctoral Fellow: *FUKUYAMA, Tatsuya*

Measurement technologies, such as live imaging and next-generation sequencers, have been the recipients of rapid development. We have entered a new era in which molecular activities and gene expression levels in living tissues can be measured at a single-cell resolution in a high throughput manner. Our research group aims to elucidate the theoretical logic of dynamic living systems from such data by combining mathematical modeling and machine learning. The research topics in our group are diverse and cover subcellular scale phenomena as well as cellular and tissue level phenomena.

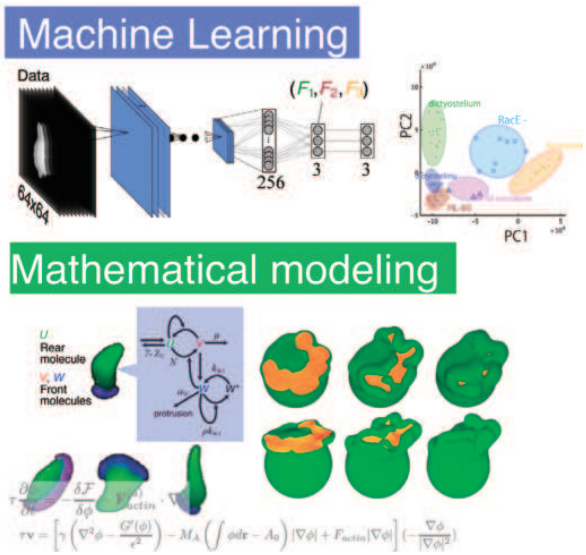


Figure 1. Researches combining machine learning and mathematical modeling.

I. Simulations of amoeboid cells

We conducted mathematical modeling of the cell shape dynamics of single cells. Migrating cells take on a variety of complex shapes and sometimes dynamically deform to perform important biological functions. On one hand, during the amoeboid movement of *Dictyostelium discoideum*, said movement is driven by the dynamic formation and disappearance of pseudopods at the cell front. On the other hand, in the epithelial cells of fish, a network of actin fibers called lamellipodia appear at the cell front, and migratory movements are performed while maintaining their overall shape. Using the phase-field method, we have developed a mathematical model that can describe the two-dimensional shape dynamics of these various forms of locomotion. We have also applied deep learning to extract features that quantify the shape of migrating cells, and systematically compared the results between experiments and simulations. By combining this type of modeling and data-driven analysis, it is possible to estimate which physicochemical parameters are responsible for the differences in cell shapes due to cell type and mutation.

We also performed three-dimensional modeling of cell deformation in micropinocytosis. Macropinocytosis refers to the nonspecific uptake of extracellular fluid, which plays a ubiquitous role in cell proliferation, immune surveillance, and virus invasion. Although this phenomenon is widespread, it is still unclear how the cup-shaped cell membrane is initially formed. We demonstrated that the protrusive force localized to the edge of the patches can give rise to a self-enclosing cup structure, without further assumptions of local bending or contraction. Furthermore, our model exhibits a variety of cup morphologies self-organized via a common mutually-dependent process of reaction-diffusion and membrane deformation.

II. Mathematical Model of Microbial Symbiosis via Metabolite Leakage

In microbial ecosystems, a wide variety of microbial species sometimes can coexist even when there is only one niche (*e.g.*, one nutrient supply). In recent years, it has been widely stated that nutrient symbiosis via metabolite leakage. Said leakage is caused by cross-feeding, a phenomenon in which a cell leaks metabolites while simultaneously utilizing another metabolites leaked from another cell, and is important for the formation of complex ecosystems. However, it is unclear why cells secrete metabolites in the first place. It makes sense if it is to dispose of unnecessary waste, but sometimes cells also secrete metabolites that are supposed to be essential for their growth.

Based on analytical and numerical calculations, we have illustrated that if the intracellular metabolism includes multibody (*e.g.*, catalytic) reactions, leakage of essential metabolites can promote the leaking cell's growth. We have also demonstrated that mutualistic relationships among diverse species can be established as a result of cell-level adaptation of metabolite leakage; each species cross-feeds others by secreting essential metabolites for their own benefit, which are usefully consumed by others, in a manner reminiscent of gift giving. In this case, the exchange of metabolites becomes entangled, which in turn leads to the coexistence of diverse microbes. The resultant ecosystems become resilient against external perturbations including the removal of each coexisting species.

III. Mathematical Model of collective cell migration

Active matter physics, which is the study of collective motions such as flocks of birds, has been well studied so far in biology and physics. Considering the motion of a cell population in terms of active matter, the cells differ significantly from existing active matter models in that the shape of individual cells can be deformed. How such deformability affects cell collective migration is not fully understood.

This study proposes a mathematical model based on the Fourier series expansion of cell contours to describe cell shape. The shape is described as a superposition of Fourier modes, and cell deformation is described as the dynamics of

each Fourier coefficient. Unlike the cell-vertex model that describes epithelial cells, the proposed model can describe various shapes other than polygons. We performed a numerical analysis of a situation in which self-propelled cells with only exclusion volume effects are densely packed using the proposed model. We found that the cell population exhibits a solidification-fluidization transition with only a change in cell softness.

Publication List:

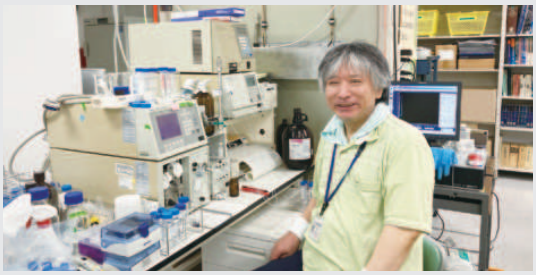
〔**Original papers**〕

- Honda, G., Saito, N., Fujimori, T., Hashimura, H., Nakamura, M.J., Nakajima, A., and Sawai, S. (2021). Microtopographical guidance of macropinocytic signaling patches. Proc. Natl. Acad. Sci. U.S.A. *118*, e2110281118. DOI: 10.1073/pnas.2110281118
- Imoto, D., Saito, N., Nakajima, A., Honda, G., Ishida, M., Sugita, T., Ishihara, S., Katagiri, K., Okimura, C., Iwadate, Y., and Sawai, S. (2021). Comparative mapping of crawling-cell morphodynamics in deep learning-based feature space. PLoS Comput. Biol. *17*, e1009237. DOI: 10.1371/journal.pcbi.1009237
- Saito, N., and Sawai, S. (2021). Three-dimensional morphodynamic simulations of macropinocytic cups. iScience *24*, 103087. DOI: 10.1016/j.isci.2021.103087
- Yamagishi, J.F., Saito, N., and Kaneko, K. (2021). Adaptation of metabolite leakiness leads to symbiotic chemical exchange and to a resilient microbial ecosystem. PLoS Comput. Biol. *17*, e1009143. DOI: 10.1371/journal.pcbi.1009143

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. Some gonadotropic hormones have been found in invertebrate species. 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.

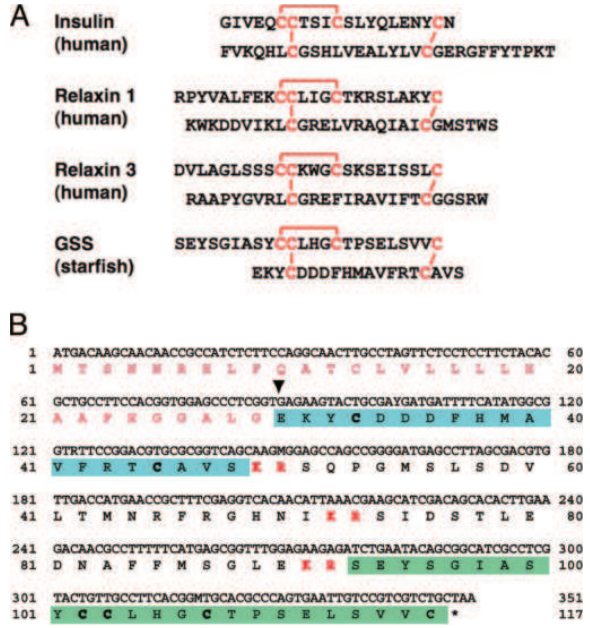


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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 and Associate Prof. Kurita'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 them, named cubifrin, which is an NGIWY-amide peptide, is in the sea cucumber *Aposticopus japonicus*.

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 in a chemical synthetic manner to provide to collaborating researchers for biological assays.

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

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

Publication List:

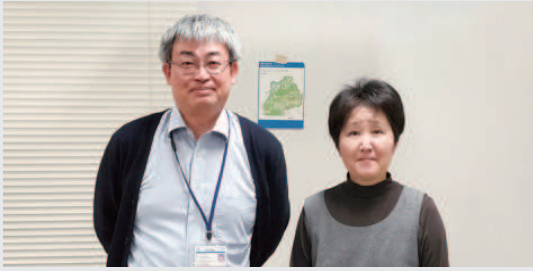
〔Original papers〕

- Nishiike, Y., Miyazoe, D., Togawa, R., Yokoyama Keiko and Nakasone, K., Miyata, M., Kikuchi, Y., Kamei, Y., Todo, T., Ishikawa-Fujiwara, T., Ohno, K., Usami, T., Nagahama, Y., and Okubo, K. (2021). Estrogen receptor 2b is the major determinant of sex-typical mating behavior and sexual preference in medaka. Curr. Biol. *31*, 1699-1710.e6. DOI: 10.1016/j.cub.2021.01.089
- Yuhi, T., Nishimiya, O., Ohno, K., Takita, A., Inoguchi, T., Ura, K., and Takagi, Y. (2022). Cloning of cDNA encoding a newly recognized apolipoprotein-like protein and its expression in the northern sea urchin *Mesocentrotus nudus*. Fish. Sci. *88*, 259–273. DOI: 10.1007/s12562-022-01584-3

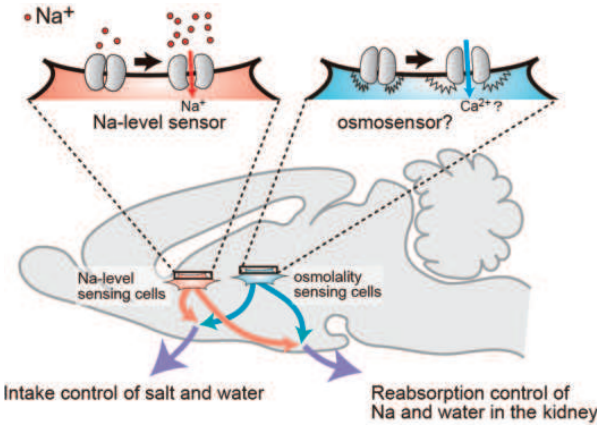
LABORATORY OF BIOLOGICAL DIVERSITY

SAKUTA Group

Assistant Professor: *SAKUTA, Hiraki*
Technical Assistant : *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.



Visual overview: Two postulated systems for sensing body-fluid conditions

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 demonstrated that Na_x signals are also involved in the control of water intake behavior. 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 epoxyeicosatrienoic acids (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.

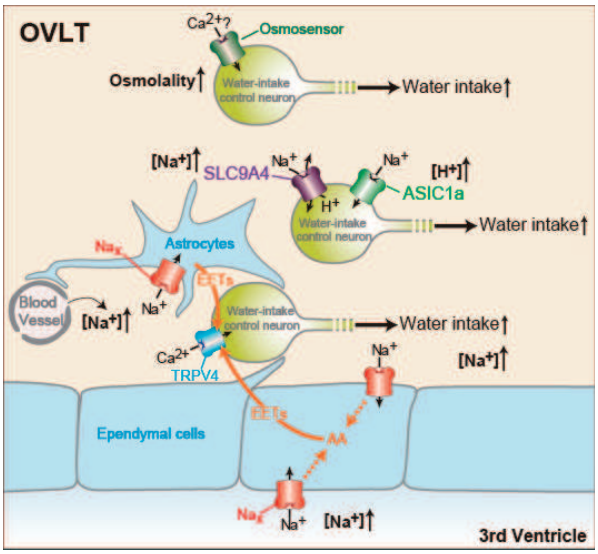


Figure 1. Schematic drawings of sensing mechanisms of body-fluid conditions in the OVLT responsible for the induction of water intake. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

Water intake by *Na_x*-KO mice after an ICV injection of hypertonic NaCl solution was small, but still approximately half that of WT mice. It was noteworthy that this was significantly higher than that of *Na_x*-KO and WT mice after an 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 many candidates for said sensors. As a result, we revealed that SLC9A4 in the OVLT functions as a $[Na^+]$ sensor for the control of water intake behavior among these candidates, and that the signaling pathway originating from this sensor is independent of the Na_x/TRPV4 pathway, another $[Na^+]$-sensing pathway for the control of water intake (Figure 1). Our experimental results suggested that SLC9A4-positive neurons are activated via ASIC1a in a H^+-dependent manner. Our experimental results also revealed that water intake induced by the increase in $[Na^+]$ in CSF was completely lost in *slc9a4*-knockdown *Na_x*-KO mice. Thus, water intake induced by $[Na^+]$ increases in body fluids may be explained by the Na_x/TRPV4 and SLC9A4/ASIC1a pathways. In addition to these two $[Na^+]$-dependent pathways that induce water intake, another signaling pathway originating from the osmosensor may be independently involved in the induction of water intake. We are now examining the functional roles of remaining candidates in water intake to clarify the osmosensing system.

LABORATORY OF BIOLOGICAL DIVERSITY

WUDARSKI Group

Specially Appointed Assistant Professor:
WUDARSKI, Jakub

Understanding the mechanisms of regeneration using transgenic flatworms and IR-LEGO

Regeneration is the process of restoring lost or damaged tissues and organs. Flatworms have long been considered as model organisms for studying regeneration; some species of planarian flatworms can even restore all their body parts from small pieces. In my research, I am using the new powerful flatworm model organism, *Macrostomum lignano*, to study how stem cells differentiate into various cell types during regeneration and how body patterning is established. The main advantage of *M. lignano* is the availability of transgenesis methods which I have developed during my PhD. It enables tracking specific cells and their progenitors during development and regeneration.

Positional control of regeneration in flatworms

Flatworms have remarkable regeneration capabilities. They are able to regrow their whole body after amputation, including their reproductive organs. They can do this thanks to a population of adult stem cells, collectively called neoblasts. One of the fascinating aspects of flatworm regeneration is the positional control of the process along the anterior-posterior axis (head-tail). How cells know where specific body parts need to be reconstructed is a question that still lacks a full answer. Our current state of knowledge is that Wnt pathway and the mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) signaling play major role in this process. However, most of the research done on flatworms is based on information inferred from experiments on gene knock-down via RNA interference (RNAi). Gene activation and overexpression studies are absent in planarians, the more common flatworm model organisms, because of the lack of transgenic methods available for these animals. I am trying to use the ERK-KTR biosensor in *Macrostomum lignano* (Fig. 1), to track ERK signaling and test the function of genes shown to be involved in positional control during growth and regeneration. I am also adapting the infrared laser evoked gene operator (IR-LEGO) technology to use with the previously established HSP20 promoter (Fig. 2). This will enable me to track the cell fate in vivo and overexpress selected genes even on a single cell level.

The research is financed by the Mistubishi Foundation grant.

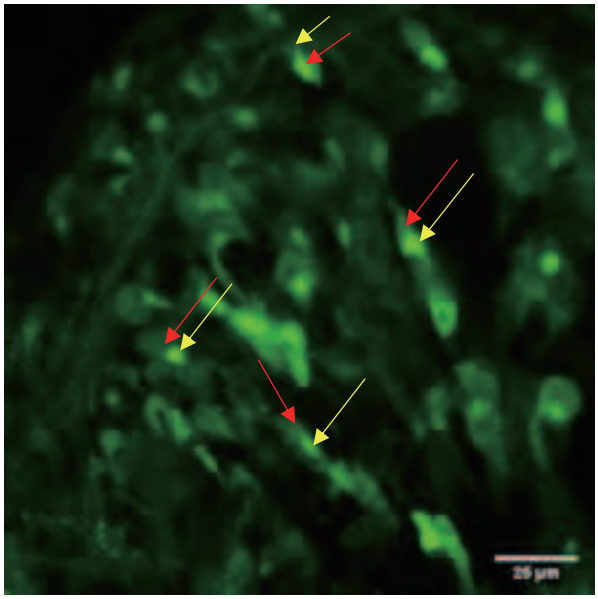


Figure 1. ERK-KTR biosensor in *Macrostomum lignano* red arrow points at the cytoplasm and yellow at the nucleus.

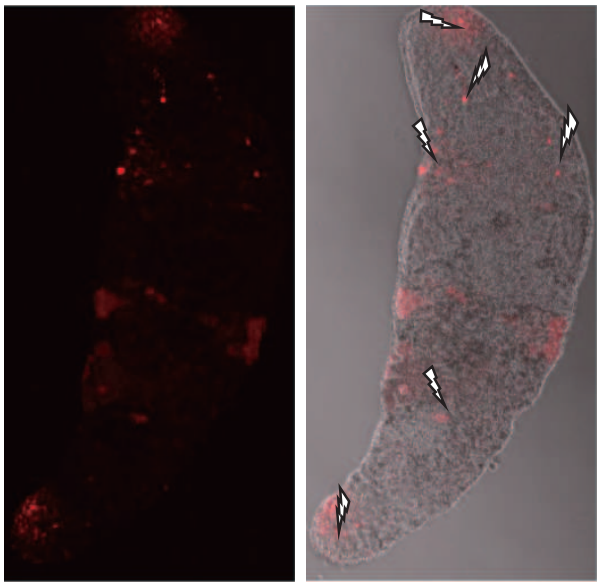


Figure 2. Expression of mScarlet under the HSP20 promoter 24 hours after induction using IR-LEGO. The lightning bolts point at the targeted sites.

Publication List:

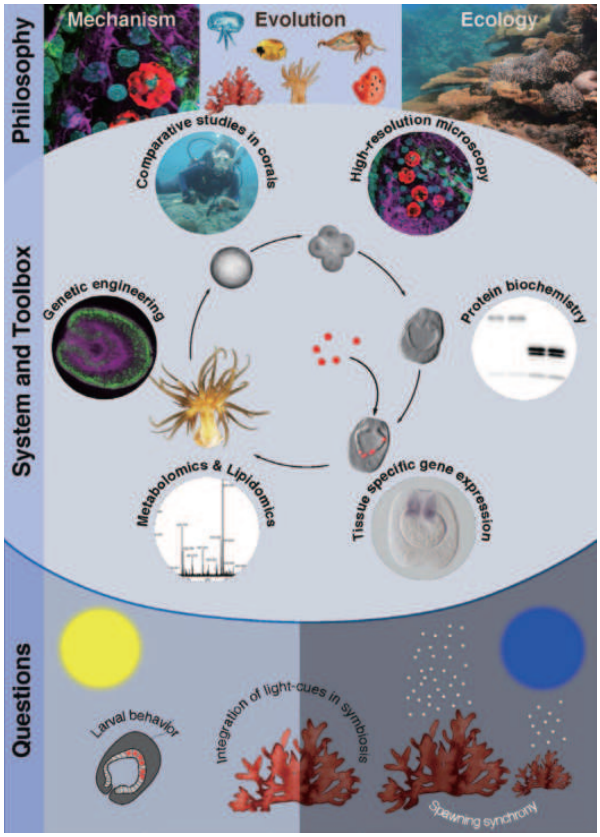
〔Original paper〕

● Ustyantsev, K., Wudarski, J., Sukhikh, I., Reinoite, F., Mouton, S., and Berezikov, E. (2021). Proof of principle for piggyBac-mediated transgenesis in the flatworm *Macrostomum lignano*. Genetics *218*, iyab076. DOI: 10.1093/genetics/iyab076

NIBB-COS International Collaboration Research Project

GUSE Group

Guest Professor: *GUSE, Annika*
Postdoctoral Fellow: *KISHIMOTO, Mariko*



Light dominates life for most organisms. On one hand, sunlight acts as a key regulator for various functions including photosynthesis and circadian clock control. Similarly, moonlight is important for synchronous gamete release in many marine animals, such as cnidarians. Cnidarians, which include corals and anemones, are basal, aquatic animals with immense ecological importance. Notably, coral reefs are the most biodiverse marine ecosystems. Their productivity depends on a functional symbiosis between reef-building corals and photosynthetic dinoflagellates of the Symbiodiniaceae family, which transfer nutrients to their coral host to provide a source of fixed carbon in oligotrophic environments.

In this COS-NIBB joint project, we aim to dissect key molecular mechanisms underlying the sensing of light in symbiotic cnidarians and how this is used in environmental adaptation. Using the sea anemone, Aiptasia sp. (*Exaiptasia diaphana*) as a model for corals, we are conducting research to reveal the molecular mechanisms of light sensing focusing on the evolutionary conserved photoreceptor "opsin". Additionally, we are aiming to establish a method of gene function analysis using genome editing technology or gene silencing techniques in said sea anemone which will in turn elucidate the mechanisms of light response by opsin. Understanding how symbiotic cnidarians perceive light to synchronize sexual reproduction and behavior will provide us with key insights into its evolution and ecology; a prerequisite to combat the decline of corals through climate change which threatens reef ecosystems worldwide.

I. Light sensing changes by symbiotic status

The gene expression of Aiptasia opsins changes depending on their symbiotic status. This suggests that symbiotic and non-symbiotic Aiptasia have different sensitivities to light. This could be due to the different opsin types and the light responses in algae.

In this study, we found that symbiotic and non-symbiotic Aiptasia have different phototaxis. Irradiation with high levels of light caused negative-phototaxis in symbiotic Aiptasia while non-symbiotic Aiptasia did not react. The symbiosis-specific negative phototaxis was dependent on the wavelength specificity, and peaked in the blue region. Thus, symbiosis with algae changes Aiptasia's light sensitivity.

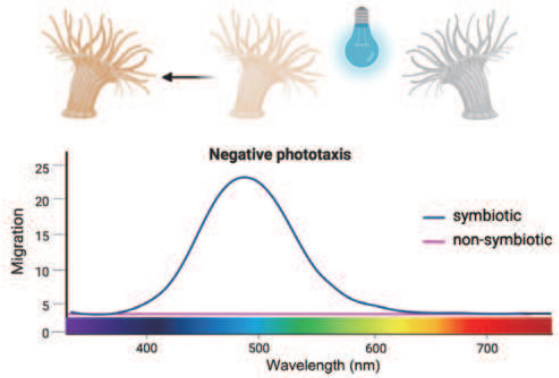


Figure 1. Wavelength specificity of negative phototaxis in symbiotic Aiptasia.

II. Functional analysis of Aiptasia opsins

Aiptasia has 18 types of opsins, and 3 of them have higher gene expression in their symbiotic state. In this study, we are aiming to analyze the light sensitivity of the distinct Aiptasia opsins using a heterologous cell culture-based assay. To date, we have successfully cloned 12/18 opsins including three symbiosis-specific ones. Using cell culture-based assays monitoring cAMP and Calcium signaling, we were able to detect a light-dependent response of these three opsins. We have determined the absorbance wavelength specificity of the three symbiosis-specific opsins. Moreover, we have found that Aiptasia anemones exhibit negative phototactic behavior in a symbiosis-dependent manner. Together, these finding provide the foundation to relating distinct opsins with light-dependent behavior including phototaxis and spawning in Aiptasia.

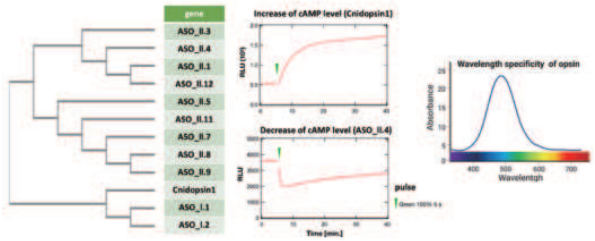


Figure 2. Functional analysis of Aiptasia opsins cloned in this study.

Publication List:

〔Original paper〕

● Urakawa, N., Nakamura, S., Kishimoto, M., Moriyama, Y., Kawano, S., Higashiyama, T., and Sasaki, N. (2022). Semi-in vitro detection of Mg^{2+}-dependent DNase that specifically digest mitochondrial nucleoids in the zygote of *Physarum polycephalum*. Sci. Rep. *12,* 2995. DOI: 10.1038/s41598-022-06920-2

LABORATORY OF BIOLOGICAL DIVERSITY

YAMASHITA AND OTSUBO Group

Specially Appointed Associate Professor
YAMASHITA, Akira

Specially Appointed Assistant Professor: *OTSUBO, Yoko*
Technical Assistant: *NAKADE, Atsuko*
Admin Support Staff: *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 group, 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.

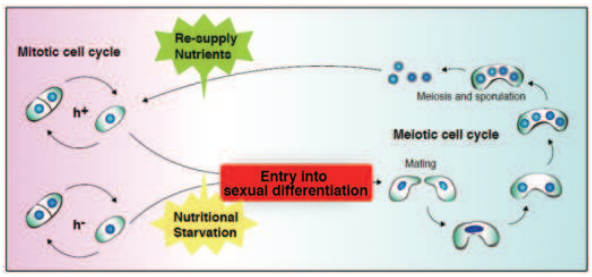


Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting 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 have focused 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 under restrictive temperature conditions. 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 several responsible genes encoded tRNA-related factors. 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). We are also studying how *S. pombe* cells respond to other types of starvation other than that of nitrogen.

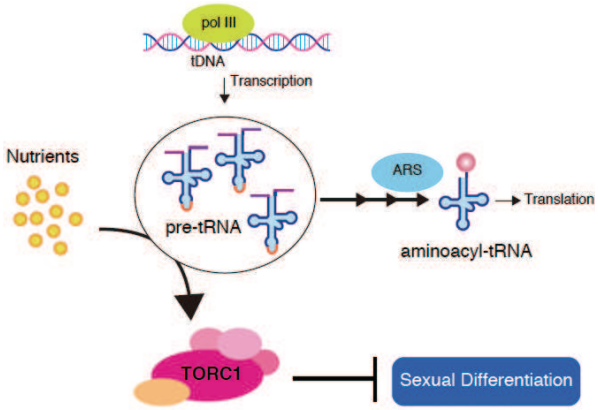


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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

The expression of hundreds of genes is upregulated during meiosis. Expression of meiotic genes is strictly regulated, since untimely expression of gametogenic genes, including meiotic genes, has a deleterious effect on somatic cells. 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 machinery. 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 referred to as 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).

We have also demonstrated that a conserved complex called MTREC (Mtl1-Red1 core) or NURS (nuclear RNA silencing) that consists of a zinc-finger protein, Red1, and an RNA helicase, Mtl1, is required for the recruitment of the nuclear exosome to Mmi1 foci.

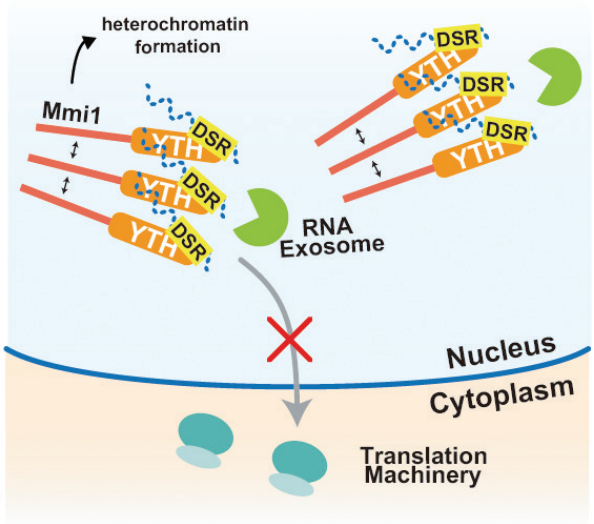


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

III. Cellular responses to atmospheric pressure plasma

Plasma, which is the fourth state of matter after solid, liquid and gas states, is a form of 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 the National Institute for Fusion Science (Figure 4).

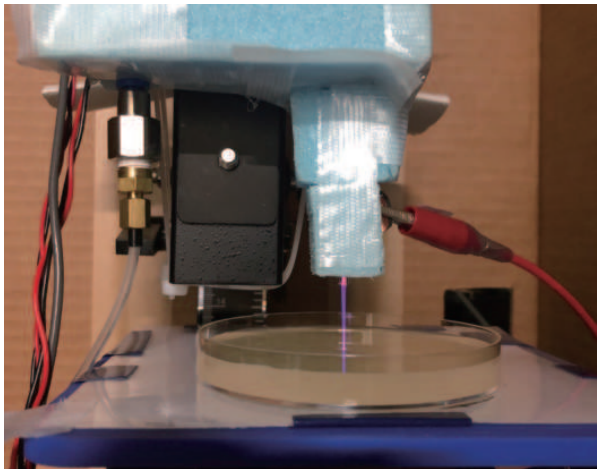


Figure 4. Direct plasma irradiation to the fission yeast *S. pombe* on an agar medium.

Publication List:

〔Original paper〕

● Ohtsuka, H., Hatta, Y., Hayashi, K., Shimasaki, T., Otsubo, Y., Ito, Y., Tsutsui, Y., Hattori, N., Yamashita, A., Murakami, H., and Aiba, H. (2022). Cdc13 (cyclin B) is degraded by autophagy under sulfur depletion in fission yeast. Autophagy Reports *1*, 51–64. DOI: 10.1080/27694127.2022.2047442

NINS ASTROBIOLOGY CENTER

The Astrobiology Center (ABC) in NISS was established in 2015 to promote interdisciplinary studies that include astronomy, earth science, and biology by organizational adaptation between National Astronomical Observatory of Japan (NAOJ) and National Institute for Basic Biology (NIBB). In the 2021 fiscal year, ABC stayed active to encourage interdisciplinary studies and grow the research community as the hub institute of astrobiology despite social constraints under the COVID-19 pandemic. ABC symposium was held online on Jan 28th, 2022, inviting speakers from diverse research fields including an observatory mission by James Webb Space Telescope. Life in the Universe Workshop was also held online on Feb 17th-18th, 2022 to present the latest results from ABC-subsidized research.

Figure 1. ABC mascot characters: "Exoplanet Friends" (Credit: Hayanon Science Manga Studio)

Three project offices in ABC are working together to find a habitable exo-planet and a sign of life on said planet. Exo-Planet Search Project Office and Astrobiology Instrument Project Office which are located in the Mitaka campus of NAOJ are preparing direct-imaging surveys of "Another Earth" by next-generation, 30m-scale telescopes. To support future observation projects, three groups from NIBB in Okazaki, the Takizawa, Johzuka, and Fujita groups, are participating in the Exo-Life Search Project Office to investigate life on Earth utilizing three different approaches: 1) assessing the biosignature of various photosynthetic organisms to predict biosignatures of hypothetical life on exoplanets, 2) evaluating the effects of cosmic radiation on living cells, and 3) elucidating mathematical principles in the formation of self-organizing structures in organisms.

LABORATORY OF BIOLOGICAL DIVERSITY

TAKIZAWA Group

Specially Appointed Associate Professor
TAKIZAWA, Kenji

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

Light adaptation mechanisms of photosynthesis

We investigated the flexible molecular mechanisms of light harvesting and following photosynthetic reactions in cooperation with the division of environmental photobiology. In photosynthesis research, we are specialized in spectroscopic analyses which can be applied to astrobiology studies.

Characterize hypothetical plants in exo-planets

One of the most plausible biosignatures detected by the exo-planet observations is a specific reflection pattern on the land surface named 'vegetation red edge' or VRE. VRE arises from strong absorption of red light by photosynthetic pigments and high reflectance of near-infrared radiation (NIR) by the developed leaf tissue structure. The wavelength position and amplitude of VRE could be different on another planet. We studied the light reflection spectrum of various plants and predicted that substantially large VRE could be observed even on the ocean planets if floating plants cover the water surface (Figure 2). We also predicted the possibility of red-sift in VRE on the planets exposed to NIR rather than visible light. We have revealed that several metal-containing pigments can be functional in reaction centers under NIR radiation conditions via quantum chemical calculations.

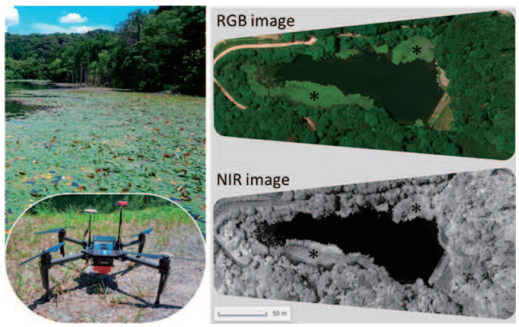


Figure 2. Remote sensing of light reflectance of floating leaf of waterlily. Light reflectance in visible light (RGB) and NIR were obtained by the drone-based multiband sensor.

Publication List:

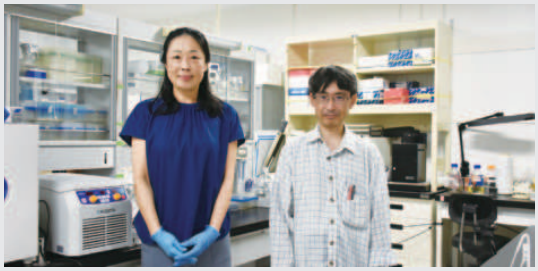
〔Original papers〕

- Komatsu, Y., and Takizawa, K. (2021). A quantum chemical study on the effects of varying the central metal in extended photosynthetic pigments. Phys. Chem. Chem. Phys. *23*, 14404–14414. DOI: 10.1039/d1cp00760b
- Pan, X., Tokutsu, R., Li, A., Takizawa, K., Song, C., Murata, K., Yamasaki, T., Liu, Z., Minagawa, J., and Li, M. (2021). Structural basis of LhcbM5-mediated state transitions in green algae. Nat. Plants *7*, 1119+. DOI: 10.1038/s41477-021-00960-8

LABORATORY OF BIOLOGICAL DIVERSITY

FUJITA Group

Assistant Professor: *FUJITA, Hironori*
Technical Assistant: *EGAWA, Akane*
TAKEKAWA, Eiko



In nature, a variety of self-organized patterns, such as the galaxy and the snowflake, are found on a wide range of spatiotemporal scales. 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 theoretical and computational approaches (Figure 1).

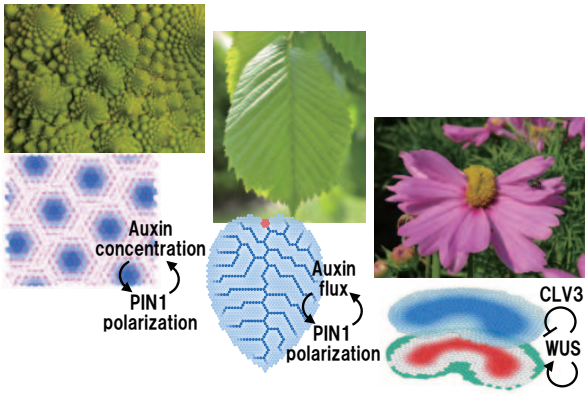


Figure 1. (Left) Phyllotaxis pattern is self-organized by the feedback regulation between auxin concentration and PIN1 polarization. (Middle) Leaf venation pattern is self-organized by the feedback regulation between auxin flux and PIN1 polarization. (Right) Shoot apical meristem (SAM) is self-organized by the interaction between WUS and CLV3.

I. Spatiotemporal self-organization of cell population

One well-known example of spatiotemporal self-organization in living systems is *Dictyostelium discoideum*, known as cellular slime mold. *D. discoideum* usually exists as a unicellular organism, but when stimulated by starvation, cells aggregate while forming a spiral pattern and develop a slug-like multicellular body. This remarkable self-organization is induced by chemotaxis to the signal molecule cAMP.

On the other hand, it is reported that *Escherichia coli*, which is a unicellular model organism, can self-organize spotted colony patterns (Figure 2B). This pattern formation is induced by the positive feedback between chemotaxis to aspartate (Asp) and Asp synthesis, and can be an excellent experimental system in the study of self-organization of cell populations. This stationary pattern of *E. coli* colony is formed under the condition of "spot inoculation", in which *E. coli* cells are inoculated in the center of soft-agar plates (Figure 2B). In order to further develop the research, we have newly developed an assay system "uniform inoculation", which causes a more dynamic self-organization in which colony spots initially emerge from the entire surface of agar medium, and they move and fuse with each other repeatedly (Figure 2C). Whereas experimental conditions in biological experiments are usually greatly restricted, the synthetic approach is a powerful research method that can control and modify the experimental conditions in a more flexible manner compared to the method used in standard experiments. Therefore, we try to apply the synthetic approach to *E. coli* to understand the principle of spatiotemporal self-organization of cell populations.

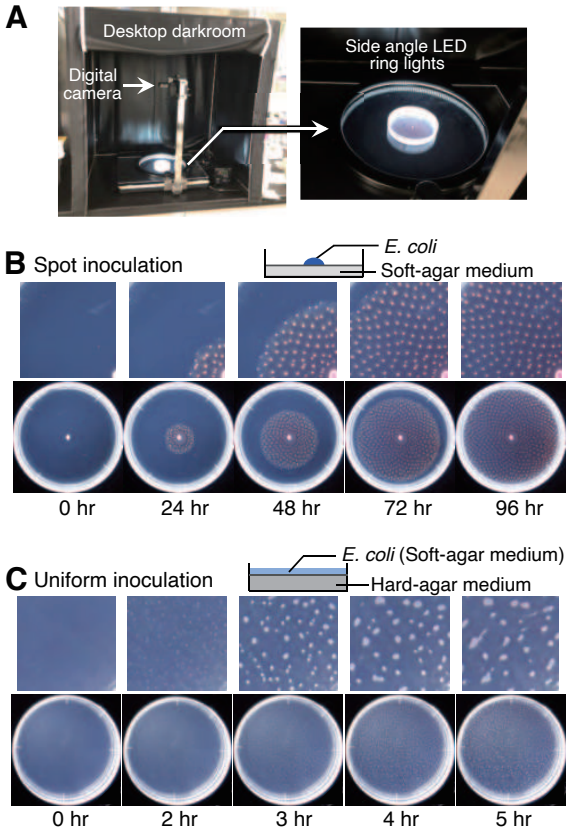


Figure 2. (A) A device system for photographs and time-lapse videos of spatiotemporal patterns of cell populations. (B) Spot inoculation; *E. coli* cells are inoculated in the center of soft-agar plates. The cell population spreads outward while forming a stable spot pattern. (C) Uniform inoculation; *E. coli* cells are evenly inoculated in soft-agar medium. The cell population shows a dynamic spatiotemporal self-organization in which colony spots initially appear from the entire surface of soft-agar medium (2 hr), and they move to fuse with each other repeatedly (2–5 hr).

Publication List:

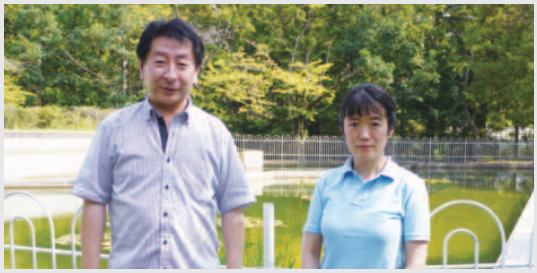
〔Original paper〕

● Kataoka, K., Fujita, H., Isa, M., Gotoh, S., Arasaki, A., Ishida, H., and Kimura, R. (2021). The human *EDAR* 370V/A polymorphism affects tooth root morphology potentially through the modification of a reaction–diffusion system. Sci. Rep. *11*, 5143. DOI: 10.1038/s41598-021-84653-4

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

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



I. Condensin-dependent chromatin folding

Chromosome condensation is a basic cellular process that ensures the faithful segregation of chromosomes in both mitosis and meiosis. 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.

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*. 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.

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.

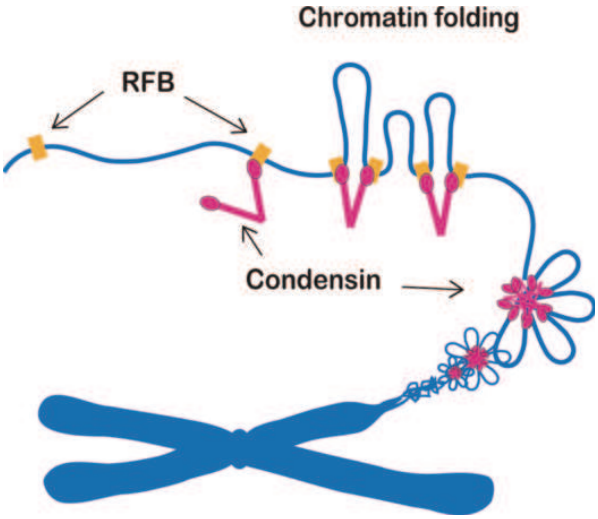


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

II. Plasma-induced cellular responses

Plasma is an ionized gas, which consist of ions and electrons and is extremely reactive. Our question is to understand the effect of the plasma on living organisms.

We have developed a new plasma irradiation device that can control the temperature to the optimal growth temperature of cells, and have developed a system to study the effects of direct plasma exposure to cells without heat stress (Figure 2). Using yeast, *S.cerevisiae*, it was found that direct plasma exposure treatment increases lethality as the processing time increases (Figure 3). Furthermore, a plasma-resistant mutant was isolated and the causative gene responsible for plasma-resistance was identified. We are studying functional analysis of the gene to understand the molecular mechanisms of the cellular responses to plasma.

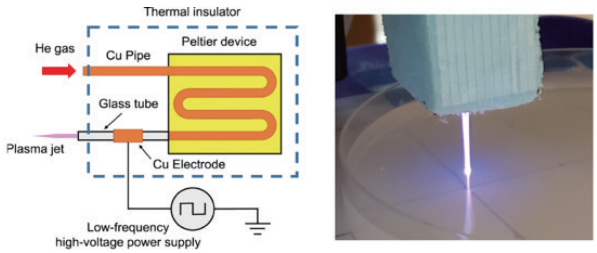


Figure 2. Schematic diagram of atomosperic pressure plasma irradiation device (left). Direct plasma irradiation experiment (right).

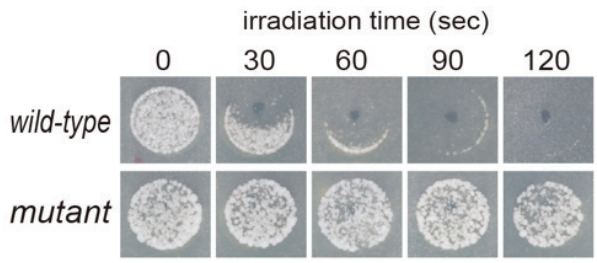


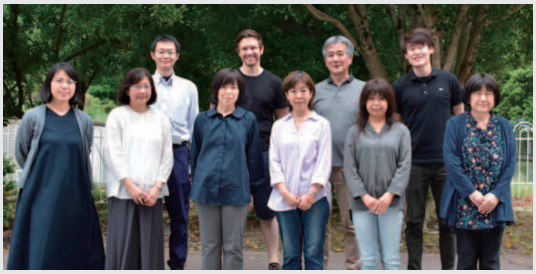
Figure 3. wild-type (upper panels) and plasma-resistant mutant (lower panels)

DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

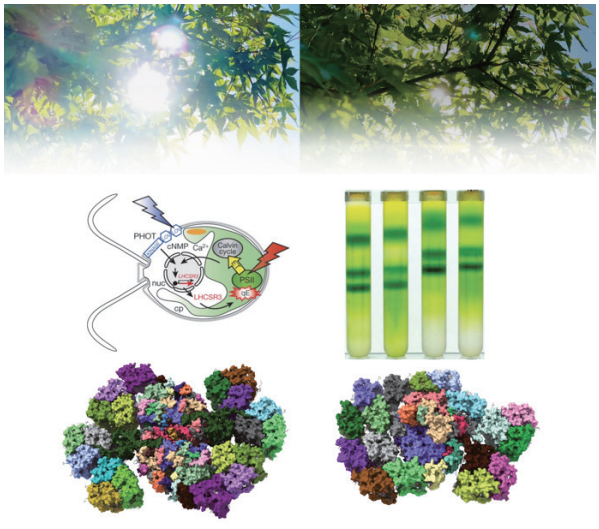
Professor
MINAGAWA, Jun

Associate Professor
YOKONO, Makio

Assistant Professor: *KIM, Eunchul*
Specially Appointed Assistant Professor: *KOSUGI, Makiko*
Technical Staff: *NODA, Chiyo*
Postdoctoral Fellow: *KAMADA, Konomi*, *ISHII, Asako*
SOKENDAI Graduate Student: *YANAKA, Ayako*
Visiting Scientist: *DANN, Marcel*
Technical Assistant: *YONEZAWA, Harumi*, *KADOWAKI, Tamaka*
Admin Support Staff: *TOYAMA, Mami*, *SUZUKI, Yuka*



Plants and algae have versatile abilities 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. 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 photosynthetic organisms, including phytoplankton and vascular plants, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.



Visual overview of this lab's work

I. Plant and Algal PSII–LHCII Supercomplexes: Structure, Evolution and Energy Transfer.

Photosynthesis is the process conducted by plants and algae to capture photons and store their energy in chemical forms. The light-harvesting, excitation transfer, charge separation and electron transfer in photosystem II (PSII) are the critical initial reactions of photosynthesis and thereby largely determine its overall efficiency. Knowledge about the architectures and assemblies of plant and green algal PSII–light harvesting complex II (LHCII) supercomplexes are rapidly accumulating (Figure 1). We made pair-wise comparative analyses between the supercomplexes from plants and green algae to gain insights about the evolution of the PSII–LHCII supercomplexes involving the peripheral small PSII subunits that might have been acquired during the evolution (Figure

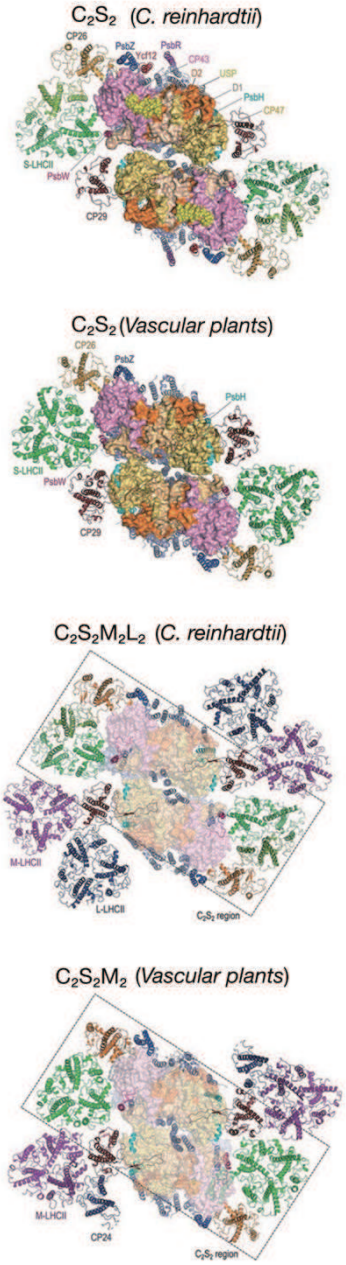


Figure 1. The overall architecture of PSII–LHCII supercomplexes from C. reinhardtii and vascular plants (C_2S_2 from Spinacia oleracea, $C_2S_2M_2$ from pea). Sheng *et al*. (2021) Plant Cell Physiol., *62*:1108-1120.

2) and about the energy transfer pathways that define their light-harvesting and photoprotective properties (Figure 3) (Sheng *et al*., *Plant Cell Physiol.*, *62*:1108-1120).

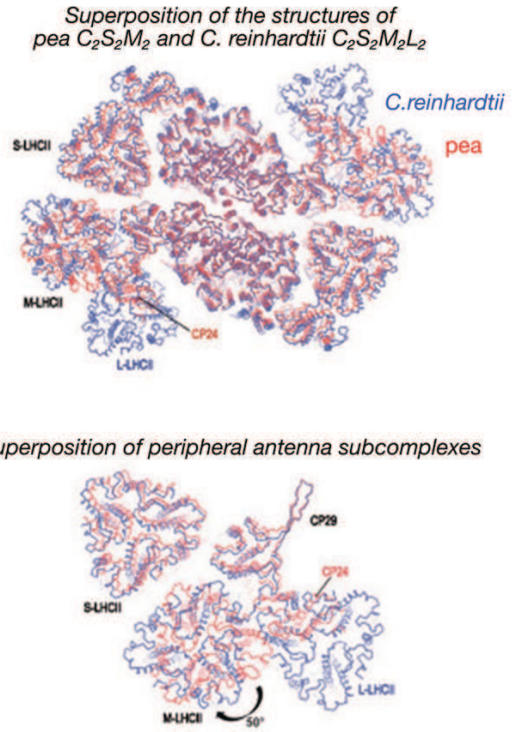


Figure 2. Superposition of green algal and plant PSII–LHCII supercomplexes and evolutionary insights from pea $C_2S_2M_2$ and *C. reinhardtii* $C_2S_2M_2L_2$ supercomplexes. Sheng *et al*. (2021) *Plant Cell Physiol.*, 62:1108-1120.

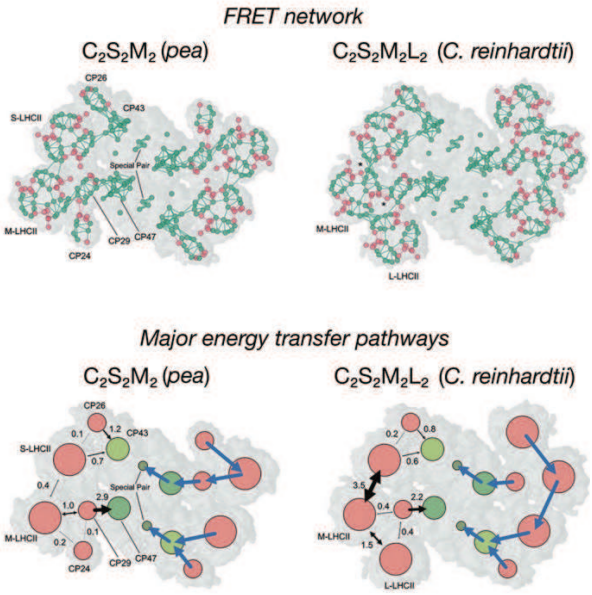


Figure 3. Förster resonance energy transfer (FRET) networks of green algal and plant PSII-LHCII supercomplexes and major energy transfer pathways. Sheng *et al*. (2021) *Plant Cell Physiol.*, *62*:1108-1120.

II. Structural basis of LhcbM5-mediated state transitions in green algae.

In green algae and plants, state transitions serve as a short-term light-acclimation process in the regulation of the light-harvesting capacity of photosystems I and II (PSI and PSII, respectively). During the process, a portion of light-harvesting complex II (LHCII) is phosphorylated, dissociated from PSII and binds with PSI to form the supercomplex PSI–LHCI–LHCII. We reported high-resolution structures of PSI–LHCI–LHCII from *Chlamydomonas reinhardtii*, revealing the mechanism of assembly between the PSI–LHCI complex and two phosphorylated LHCII trimers containing all four types of LhcbM protein (Figure 4). Two specific LhcbM isoforms, namely LhcbM1 and LhcbM5, directly interact with the PSI core through their phosphorylated amino terminal regions. Furthermore, biochemical and functional studies on mutant strains lacking either LhcbM1 or LhcbM5 indicate that only LhcbM5 is indispensable in supercomplex formation. The results unravel the specific interactions and potential excitation energy transfer routes between green algal PSI and two phosphorylated LHCIIs (Pan *et al*., *Nat. Plants*, *7*:1119-1131).

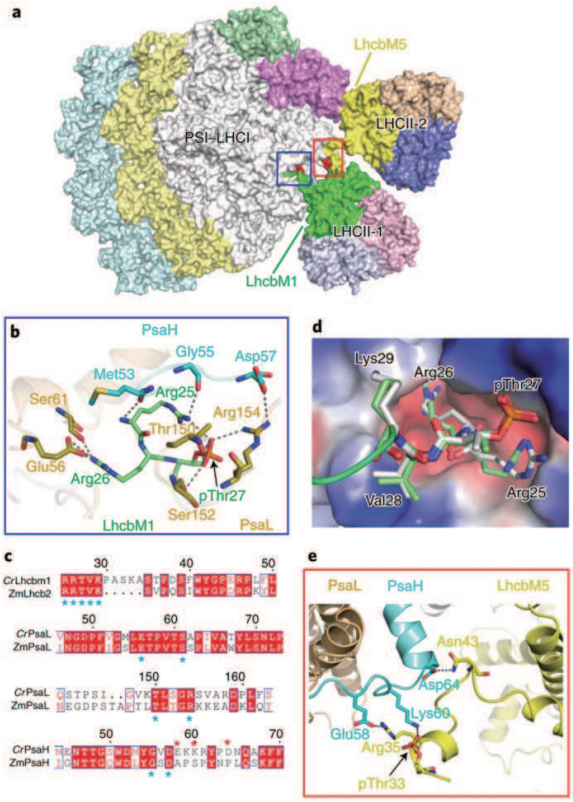


Figure 4. Surface representation of the *Cr*PSI–LHCI–LHCII supercomplex from the *pph1;pbcp* mutant strain, with pThr residues from LhcbM1 and LhcbM5 highlighted in red. **b**, Detailed interaction between the N-terminal region of LhcbM1 and PSI core subunits PsaH and PsaL. **c**, Sequence alignment of the interaction-related regions of LhcbM1, PsaL and PsaH from *C. reinhardtii* (*Cr*) with Lhcb2, PsaL and PsaH from Zm, respectively. Conserved and variant residues involved in intersubunit interactions are indicated by blue and red asterisks, respectively. **d**, Structural comparison of N-terminal five residues (shown as sticks) of *Cr*LhcbM1 (carbon atoms coloured green) and *Zm*Lhcb2 (carbon atoms coloured white). The PSI core is shown in electrostatic surface mode with red and blue representing acidic and basic regions, respectively. **e**, Detailed interactions between the N-terminal region of LhcbM5 and the PSI core subunit PsaH. Hydrogen bond interactions are shown as black dashed lines. Pan *et al*. (2021) *Nat. Plants*, *7*:1119-1131.

Publication List:

〔Original Papers〕

- Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., and Shigenobu, S. (2021). Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, Plakobranchus ocellatus. eLife *10*, e60176. DOI: 10.7554/eLife.60176
- Morishita, J., Tokutsu, R., Minagawa, J., Hisabori, T., and Wakabayashi, K. (2021). Characterization of Chlamydomonas reinhardtii Mutants That Exhibit Strong Positive Phototaxis. Plants *10*, 1483. DOI: 10.3390/plants10071483
- Pan, X., Tokutsu, R., Li, A., Takizawa, K., Song, C., Murata, K., Yamasaki, T., Liu, Z., Minagawa, J., and Li, M. (2021). Structural basis of LhcbM5-mediated state transitions in green algae. Nat. Plants *7*, 1119+. DOI: 10.1038/s41477-021-00960-8
- Tanno, A., Tokutsu, R., Arakaki, Y., Ueki, N., Minagawa, J., Yoshimura, K., Hisabori, T., Nozaki, H., and Wakabayashi, K.-I. (2021). The four-celled Volvocales green alga Tetrabaena socialis exhibits weak photobehavior and high-photoprotection ability. PLoS One *16*, e0259138. DOI: 10.1371/journal.pone.0259138
- Tokutsu, R., Fujimura-Kamada, K., Yamasaki, T., Okajima, K., and Minagawa, J. (2021). UV-A/B radiation rapidly activates photoprotective mechanisms in Chlamydomonas reinhardtii. Plant Physiol. *185*, 1894–1902. DOI: 10.1093/plphys/kiab004
- Zhang, X.J., Fujita, Y., Tokutsu, R., Minagawa, J., Ye, S., and Shibata, Y. (2021). High-Speed Excitation-Spectral Microscopy Uncovers In Situ Rearrangement of Light-Harvesting Apparatus in Chlamydomonas during State Transitions at Submicron Precision. Plant Cell Physiol. *62*, 872–882. DOI: 10.1093/pcp/pcab047

〔Review Article〕

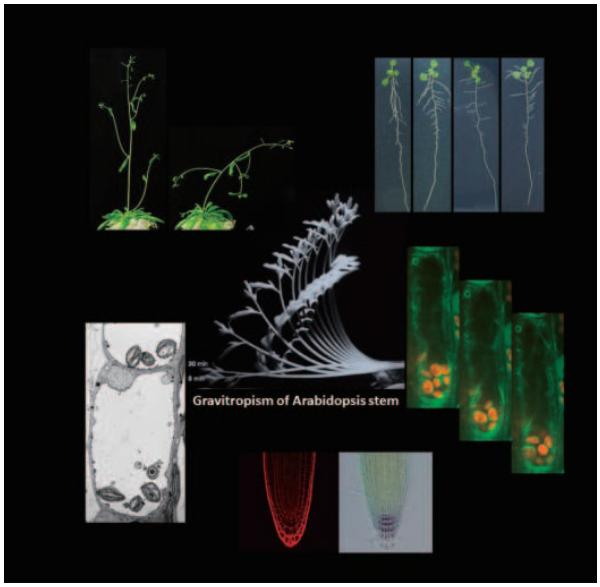
- Hippler, M., Minagawa, J., and Takahashi, Y. (2021). Photosynthesis and Chloroplast Regulation-Balancing Photosynthesis and Photoprotection under Changing Environments. Plant Cell Physiol. *62*, 1059–1062. DOI: 10.1093/pcp/pcab139
- Sheng, X., Liu, Z., Kim, E., Minagawa, J. (2021). Plant and algal PSII-LHCII supercomplexes: structure, evolution and energy transfer. Plant Cell Physiol *62*: 1108-1120. DOI: 10.1093/pcp/pcab072

DIVISION OF PLANT ENVIRONMENTAL RESPONSES

Professor
MORITA, Miyo T.

Assistant Professor: *NISHIMURA, Takeshi*
SHIKATA, Hiromasa
Specially Appointed Assistant Professor:
KAWAMOTO, Nozomi
Research Staff: *TAKASE, Wakana*
Technical Assistant: *HAMADA, Mayako*
YAMADA, Yuka
SOMA, Yoriko
INOUE, Fumiko
MOTOMURA, Hiroe
Admin Support Staff: *KOJIMA, Yoko*

Plant organs have the ability to sense various vectorial stimuli such as light, humidity, touch and gravity as well as reorient their growth direction so as to be in a suitable position to survive and acclimate to their environment. Our aim is to understand the molecular mechanism of environmental responses as they pertain to gravity in the main.



Visual overview of this lab's work.

I. Molecular mechanisms of gravity sensing and signaling in gravitropism

Gravitropism is a major determinant in directing plant organ growth angles. In gravity sensing cells (statocytes), plastids accumulating starch in high-densities 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 the polar auxin transport necessary to change the direction that a given plant is growing. We are investigating the detailed molecular mechanism of gravity sensing and signaling by using the model plant *Arabidopsis thaliana*.

LAZY1 family genes have been shown to be involved in gravitropic responses in a variety of plants. *LAZY1-LIKE*(*LZY*)*2*, *LZY3*, and *LZY4* are involved in root gravitropism of Arabidopsis. Previously, we have found that LZY3-mCherry is polarly localized on the basal side of the plasma membrane in the columella cells (root statocytes) in response to inclination. This suggests the close relationship between intracellular localization of LZY and gravity sensing. To elucidate the gravity sensing mechanism, we are analyzing regions responsible for intracellular localization of LZY3-mCherry and LZY4-mSacarlet by introducing mutations. A number of transgenic lines harbouring mutated LZY-FP driven by their own promoters in mutant background are under construction.

II. Determination mechanism of gravitropic setpoint angle

Growth angles affected by gravity are known as the gravitropic setpoint angles (GSA). Many gravitropic mutants show abnormal GSA in lateral branches; meaning they produce wider growth angle phenotypes due to the likelihood of reduced gravitropism. We are trying to understand how roots' and shoots' lateral branches maintain inclined growth angles with respect to gravity.

It has been proposed that the GSA is determined by two antagonistic growth components, gravitropism and antigravitropic offset (AGO). Currently, we are characterizing Arabidopsis *TAC1* (*TILLER ANGLE CONTROL 1*) gene as a candidate to control AGO. The *TAC1* gene was originally identified by quantitative trait locus analysis in rice tiller angle control (Yu *et al*., 2007). Later, *TAC1* gene was identified in Peach tree (*Prunus persica*) as a causal gene of piller phenotype (Dardick *et al*., 2013). In our lab, *TAC1* gene was found as a down regulated gene in agravitropic mutants *eal1* and *sgr1* which lack an endodermis, a gravity sensing tissue in shoot in Arabidopsis (Taniguchi *et al*., 2017). In Arabidopsis, *tac1* mutant has a compact shoot architecture due to the upward lateral branch growth angles (Figure 1). *TAC1* encodes a protein with unknown function, but has a similarity with LAZY1 family protein. However, while LZY family proteins interact with RLD family proteins through their conserved C-terminus in LAZY1 family protein (CCL) domain and regulate gravitropism (Furutani *et al*., 2020), TAC1 does not have a CCL domain, thereby TAC1 does not interact with RLD family protein.

As well as characterizing *TAC1* gene, currently, we are also trying to isolate suppressor mutants of *lzy1 lzy2 lzy3* by a forward genetic screening.

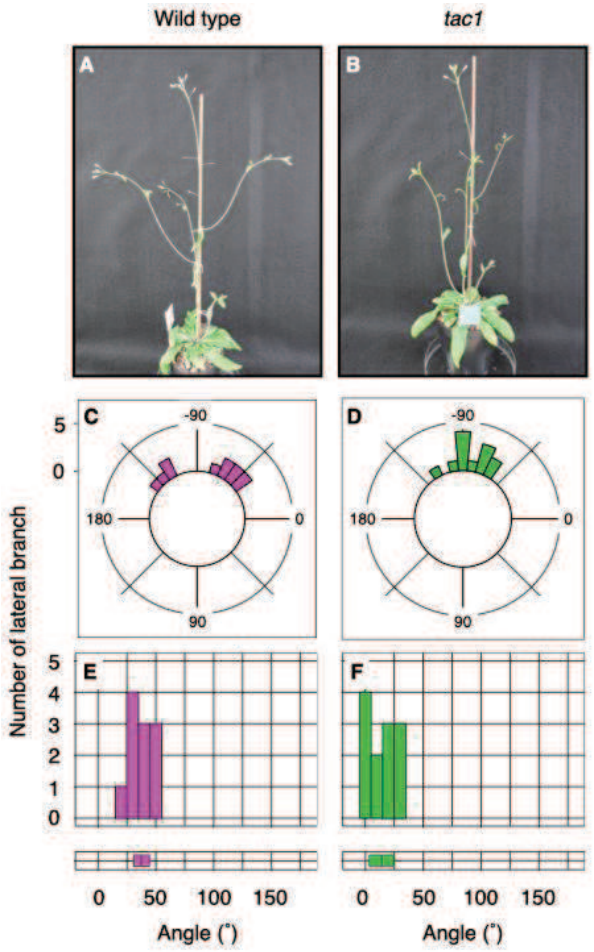


Figure 1. Growth angle phenotype of shoot lateral branches. Overall shoot architecture in wild type (A) and *tac1* (B). Distribution of growth direction of lateral branches in wild type (C) and *tac1* (D). Growth angles were plotted on histogram and distributions were evaluated in boxplot from wild type (E) and *tac1* (F).

III. Functional analysis of RLD (RCC1-like domain) proteins

RLD proteins were isolated as interactors of LZY. RLDs function in gravitropism and the GSA control of roots. We have also shown that *RLD1*, *RLD2*, *RLD3*, and *RLD4* redundantly function in plant development and morphogenesis, the morphological abnormality gradually becomes more severe by multiplying *rld* mutations (Figure 3). Indeed, *rld1;2;3;4* quadruple mutants displayed severe embryonic development defects due to a reduced amount and abnormal localization of PIN proteins (auxin efflux carriers) in the plasma membrane. It had been reported that the RLD1 fragment containing RCC1-like domain show guanine nucleotide exchange activity in Rab8 and Rab11. Thus, it's likely that RLD proteins regulate auxin transport through regulating membrane trafficking during root gravitropism and development.

In columella cells, RLD1 is polarly recruited to the plasma membrane by interacting with LZY3 and regulates the direction of auxin transport. The interaction between RLD1 and LZY3 occurs through the binding of the BRX domain of RLD1 to the CCL domain of LZY3.

To understand further function of RLDs, we made attempt to identify the proteins that interact with RLD using yeast two-hybrid screening. As a result, we found several proteins as candidates for RLD interactor in addition to LZYs. Among them, we further analyzed the polarity protein BASL (BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE) which controls stomatal asymmetric cell division as a collaboration with Dr. Dong and colleagues (Wang *et al*., 2021). Further analyses using yeast two-hybrid system revealed that BASL interact with the BRX domain of RLD. Developmental defects of *rld1;2;3;4* mutant phenocopy those of the severe allele of gnome mutants as previously reported (Furutani *et al*., 2020). GNOM, a guanine nucleotide exchange factor for ARF (ADP ribosylation factor), plays important roles in membrane trafficking. Dong and colleagues found that RLD physically interacts with GNOM in vitro and in vivo, and that GNOM and RLDs are required for BASL polarization. Interestingly, we found GNOM in the immunoprecipitates with LZY proteins (Furutani *et al*., 2020), implying that RLD-GNOM could be involved in LZY function in the gravity signaling.

To investigate the importance of the BRX domain for RLD function, we examined whether *RLD3p:RLD3ΔBRX-mClover3* could complement the morphological phenotype of *rld2;3;4*. Unexpectedly, RLD3ΔBRX-mClover3 recovered the morphological abnormality of *rld2;3;4* (Figure 2). Interestingly, defects in gravity response including GSA control and gravitropism were observed in *RLD3p:RLD3ΔBRX-mClover3/rld2;3;4*. Both of these indicate that RLD3 lacking the BRX domain retains the function for morphogenesis but not for gravity response in shoot. Moreover, when RLD3 was expressed under the control of the endodermis-specific ADF9 promoter in *RLD3p:RLD3ΔBRX-mClover3/rld2;3;4*, the gravity response was significantly restored, thus suggesting that RLD is involved in gravity signaling in the endodermal cells in shoot gravitropism in a manner similar to LZY. These results suggest that RLD function in several physiological events including gravitropism, morphogenesis in BRX-dependent and -independent manner.

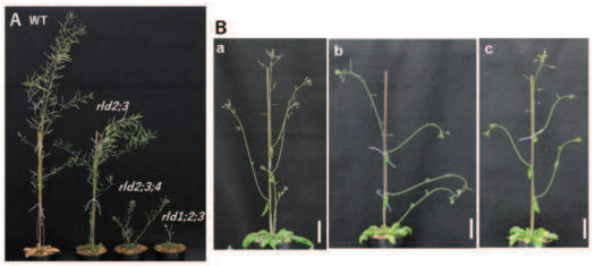


Figure 2. A. Phenotype of rld multiple mutants in mature plants. B. The growth angle of lateral shoot in Wild Type (a), RLD3p:RLD3ΔBRX-mClover3/rld2;3;4 (b), ADF9p:RLD3/RLD3p:RLD3ΔBRX-mClover3/rld2;3;4 (c).

IV. Optogenetic approaches in plant biology

Optogenetics is a powerful, biological technique to manipulate the activity and localization of proteins in living cells, thereby understanding their function. Many optogenetic tools have been developed for the past three decades, however there are few applications in plant biology because of bio-

logical and technical limitations; 1) plants use light for photosynthesis or as their development cues and 2) most of the tools are developed from plant light-sensitive proteins, in which light stimulating the tools may affect plant growth and development in an undesirable manner. Even so, optogenetic approaches are worth introducing into plant biology because they can control proteins of interest with a higher spatiotemporal resolution than those of conventional techniques like gene-inducible system and protein-uncaging with chemicals. We have been trying to optically control plant protein kinases, particularly belonging to AGC kinase family, which regulate fundamental cellular processes in plant growth and development, such as transport of plant hormone auxin and cell growth. We chose a strategy to control the kinase activity directly by light, which can be controllable at the subcellular level. To carry out it, we fused light-responsive proteins/modules to the kinases and analyzed their responsibility to light. One of successful tools possess a blue light-sensitive LOV2 domain from plant photoreceptor phototropin and its interacting motif that we identified to negatively regulate AGC kinase activity by blue light. By using this tool, we succeeded to develop a light-sensitive kinase that directly regulates the auxin transporter PINs, which is proven at least by a heterogenous expression system with *Xenopus* oocytes. It is expected that this tool will be a breakthrough technique in plant biology to dissect lots of biological events mediated by auxin (Figure 3).

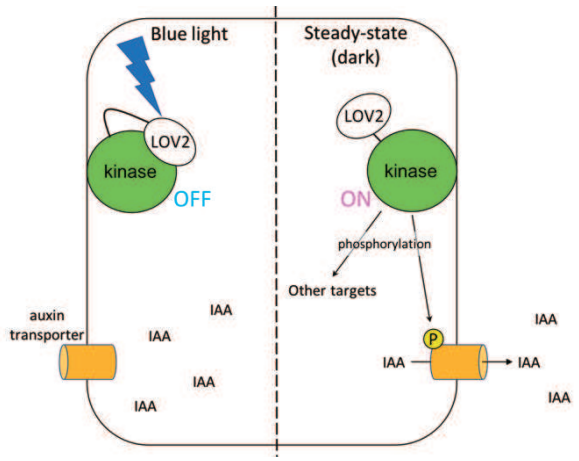


Figure 3. Schematic illustration of the light-sensitive AGC kinase. In the dark, the chimeric AGC kinase is active to phosphorylate its target proteins such as auxin transporter PINs (right). PINs are activated by the phosphorylation for efflux transport of indole-3-acetic acid (IAA). Upon blue light irradiation, the kinase activity can be reduced due to the blue light-induced conformational change (left).

Publication List:

〔Original paper〕

- Fujihara, R., Uchida, N., Tameshige, T., Kawamoto, N., Hotokezaka, Y., Higaki, T., Simon, R., Torii, K.U., Tasaka, M., and Aida, M. (2021). The boundary-expressed *EPIDERMAL PATTERNING FACTOR-LIKE2* gene encoding a signaling peptide promotes cotyledon growth during *Arabidopsis thaliana* embryogenesis. Plant Biotechnol. *38*, 317–322. DOI: 10.5511/plantbiotechnology.21.0508a
- Kunita, I., Morita, M.T., Toda, M., and Higaki, T. (2021). A three-dimensional scanning system for digital archiving and quantitative evaluation of Arabidopsis plant architectures. Plant Cell Physiol. *62*, 1975–1982. DOI: 10.1093/pcp/pcab068
- Wang, L., Li, D., Yang, K., Guo, X., Bian, C., Nishimura, T., Le, J., Morita, M.T., Bergmann, D.C., and Dong, J. (2022). Connected function of PRAF/RLD and GNOM in membrane trafficking controls intrinsic cell polarity in plants. Nat. Commun. *13*, 7. DOI: 10.1038/s41467-021-27748-w

〔Review article〕

- Furutani, M., and Morita, M.T. (2021). LAZY1-LIKE-mediated gravity signaling pathway in root gravitropic set-point angle control. Plant Physiol. *187*, 1087–1095. DOI: 10.1093/plphys/kiab219

LABORATORY OF GENOME INFORMATICS

Associate Professor
UCHIYAMA, Ikuo

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, in order 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, https://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 tables on the fly by using a specified set of organisms. MBGD also has precalculated ortholog tables for each major taxonomic group, and provides several viewing modes to display the entirety of each ortholog table. For 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.

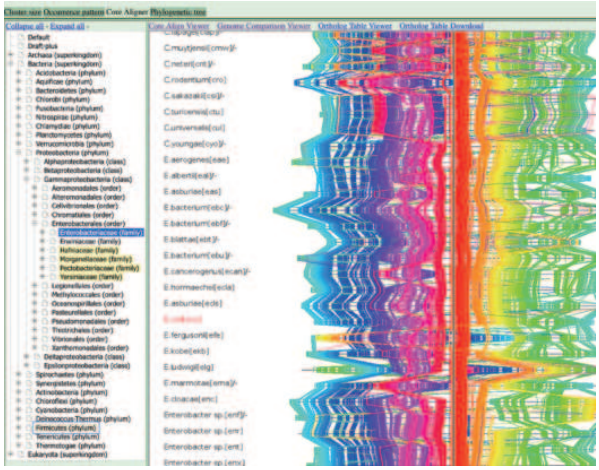


Figure 1. Comparative genome map among the family *Enterobacteriaceae* on the basis of the core genome alignment constructed using a novel implementation of the CoreAligner program. The color is assigned according to the gene order in *Escherichia coli*.

We continue to update the database and MBGD now contains 15397 genomes, including 14786 bacteria, 336 archaea, and 275 eukaryota. These data sets are classified based on the hierarchical ortholog classification strategy described in the section below. In addition, in the latest version of MBGD, we improved several functionalities including a new display of the core genome alignment (Figure 1) and a novel interface for the MyMBGD mode.

As an application software for analyzing novel genome sequences based on the database, we have developed a tool to predict the functional potential of novel microbial genomes through orthology assignment based on the MBGD ortholog table. To evaluate the metabolic potential of the query genome from this assignment, we utilized the Genomaple software to calculate the module completion ratio for each KEGG Module entry (in collaboration with Dr. Takami, Univ Tokyo). The result is displayed on a module completion table where a user can compare the presence or absence of functional modules among specified organisms.

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).

To create more comprehensive ortholog classification, 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.

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 used to construct the standard ortholog table covering all the representative genomes stored in MBGD.

Domain-level classification is a unique feature within our ortholog classification system. In particular, this data is considered suitable for analyzing domain fusion events that have occurred during evolution. By using the domain-level ortholog grouping data combined with taxonomic and functional information, we are trying to elucidate when and in what kind of genes domain fusion events frequently occur during evolution, and how the complexity of the "domain-fusion network" can be associated with the phenotypic traits in each organism.

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 five 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. The resulting data is available through the database DB-HABs (https://hab.nibb.ac.jp).

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 the Orthology Ontology (ORTH) and described the ortholog information in MBGD in the form of the Resource Description Framework (RDF).

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, we are now focusing on the remaining part of the genomes (non-core genomes) that is more directly linked to the within-species genome diversity. 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. We applied the method to the sets of genomes of prokaryotic species stored in MBGD and developed a database for analyzing their non-core genomes. Based on the database, we found that the resulting conserved clusters frequently correspond to known mobile genetic elements and/or have sequence features common to known genomic islands.

Publication List:

〔Original Papers〕

- Kumazawa, M., Nishide, H., Nagao, R., Inoue-Kashino, N., Shen, J.-R., Nakano, T., Uchiyama, I., Kashino, Y., and Ifuku, K. (2022). Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from *Chaetoceros gracilis* and Lhcq/Lhcf diversity. Physiol. Plant. *174*, e13598. DOI: 10.1111/ppl.13598
- Okubo, T., Toyoda, A., Fukuhara, K., Uchiyama, I., Harigaya, Y., Kuroiwa, M., Suzuki, T., Murakami, Y., Suwa, Y., and Takami, H. (2021). The physiological potential of anammox bacteria as revealed by their core genome structure. DNA Res. *28*, dsaa028. DOI: 10.1093/dnares/dsaa028

〔Review Article〕

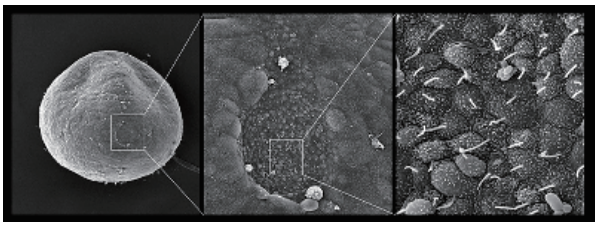
- Linard, B., Ebersberger, I., McGlynn, S.E., Glover, N., Mochizuki, T., Patricio, M., Lecompte, O., Nevers, Y., QFO Consortium, Thomas, P.D., Gabaldon, T., Sonnhammer, E., Dessimoz, C., and Uchiyama, I. (2021). Ten Years of Collaborative Progress in the Quest for Orthologs. Mol. Biol. Evol. *38*, 3033–3045. DOI: 10.1093/molbev/msab098

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

Specially Appointed Assistant Professor: *YOKE, Hiroshi*
Postdoctoral Fellow: *TANIGUCHI, Atsushi*
Technical Assistant: *ISHIBASHI, Tomoko*

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



Visual overview of this lab's work.

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.

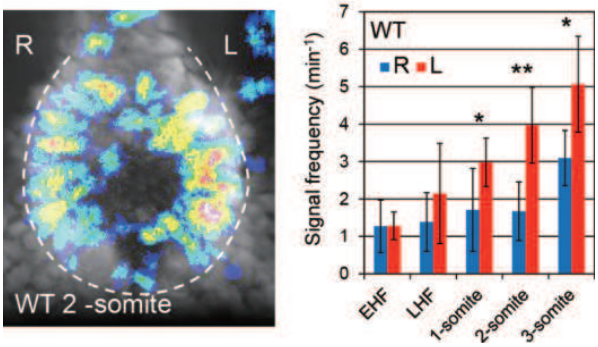


Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

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.

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.

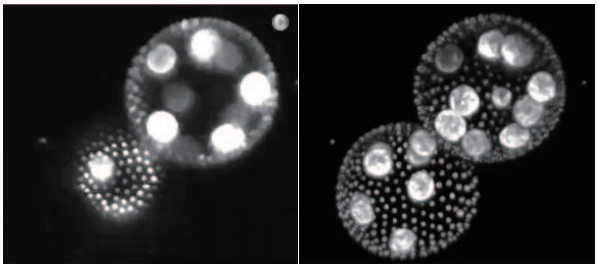


Figure 2. Images of floating volvox taken by ezDSLM with ETL. Left: Single optical section. Right: Maximum intensity projection.

Publication List:

〔Original papers〕

- Hirata, Y., Matsuo, M., Kurihara, K., Suzuki, K., Nonaka, S., and Sugawara, T. (2021). Colocalization Analysis of Lipo-Deoxyribozyme Consisting of DNA and Protic Catalysts in a Vesicle-Based Protocellular Membrane Investigated by Confocal Microscopy. Life (Basel) *11*, 1364. DOI: 10.3390/life11121364
- Ohmura, T., Nishigami, Y., Taniguchi, A., Nonaka, S., Ishikawa, T., and Ichikawa, M. (2021). Near-wall rheotaxis of the ciliate Tetrahymena induced by the kinesthetic sensing of cilia. Sci. Adv. *7*, eabi5878. DOI: 10.1126/sciadv.abi5878

LABORATORY FOR BIOTHERMOLOGY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

Specially Appointed Assistant Professor: *SAKAMOTO, Joe*
Postdoctoral Fellow: *TOMOI, Takumi*
JSPS Postdoctoral Fellow: *SUZUKI, Miyuki*
Technical Assistant: *KINOSHITA, Chie*

Temperature is an important parameter for living organisms. Cell activity is affected by temperature since the reaction ratio and stability of molecules, especially proteins in cells, depends on it. The environmental temperature which organisms function in vary, and the biological system that regulates body temperature, homeostasis, differs among various organisms. Furthermore, temperatures in either cells or at the molecular level have not been widely discussed to date. Nano-scale thermometers utilizing fluorescent proteins and dyes have been developed by numerous research groups including our own, 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 involved in single cells remain unclear. Therefore, our group is embarking upon a new research field, biothermology, by investigating the nature of temperature in living organisms ranging from nano to macro.

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 an objective lens on a microscope This technology was originally developed for laser induced gene expression in the targeting of single-cells through the heat shock response, 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

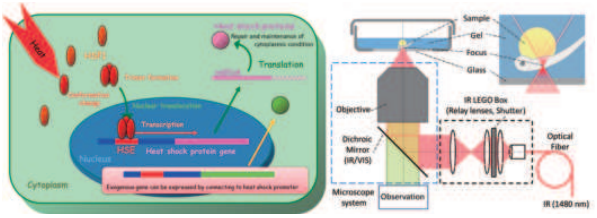


Figure 1. Schematic illustration of heat shock response in cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

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 responsiveness 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 as well as considering its meaning from a biological standpoint.

Furthermore, our group is trying to improve the IR-LEGO technique. We initially applied this system to many organisms including animals (*i.e*. medaka, nematode, flies and frogs) and plants (*i.e*. *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 the 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 utilizing fluorescent proteins. By using these transgenic lines, evidence was provided that indicated that exoskeletal tissues in the trunk region came from the mesoderm (Shimada *et al*, Nat Commun. 2013).

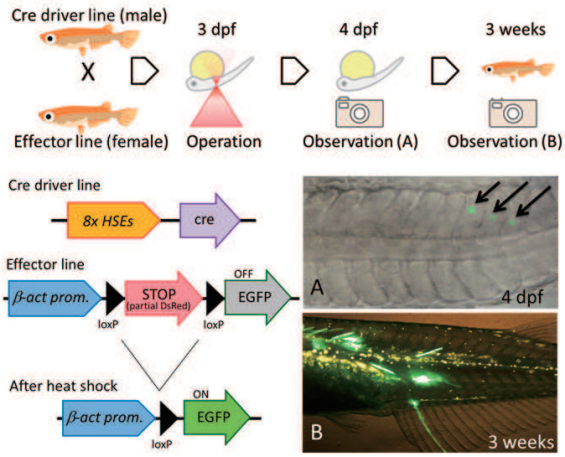


Figure 2. Long-term gene expression using the cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

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. The HSF1 substitution provides us with various types of significant information and evidence related to molecular evolution and adaptation to environmental temperature. Thus, this is how 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.

Publication List:

〔Original papers〕

- Matsuo, M., Kamei, Y., and Fukamachi, S. (2021). Behavioural red-light sensitivity in fish according to the optomotor response. R. Soc. Open Sci. *8,* 210415. DOI: 10.1098/rsos.210415
- Ogino, H., Kamei, Y., Hayashi, T., Sakamoto, J., Suzuki, M., and Igawa, T. (2021). Invention sharing is the mother of developmental biology PREFACE. Dev. Growth Differ. *63*, 395–396. DOI: 10.1111/dgd.12755
- Tokanai, K., Kamei, Y., and Minokawa, T. (2021). An easy and rapid staining method for confocal microscopic observation and reconstruction of three-dimensional images of echinoderm larvae and juveniles. Dev. Growth Differ. *63*, 478–487. DOI: 10.1111/dgd.12758
- Wu, D., Arakawa, H., Fujita, A., Hashimoto, H., Hibi, M., Naruse, K., Kamei, Y., Sato, C., and Kitajima, K. (2021). A point-mutation in the C-domain of CMP-sialic acid synthetase leads to lethality of medaka due to protein insolubility. Sci. Rep. *11,* 23211. DOI: 10.1038/s41598-021-01715-3

NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities support basic biological research conducted 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
	MORI, Syogo
Technical Assistant:	*ASAO, Hisayo*
	AKITA, Asaka
	MATSUMOTO, Miwako
Admin Support Staff:	*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 by providing close consultation and training.

In 2021, we still suffered from the effects of the COVID-19 pandemic that hampered research activities worldwide. While we operated our facility placing the highest priority on users' safety, we provided remote support for the facilities' users and online communication with collaborators to sustain research projects. Such efforts resulted in 11 co-authored papers being 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. We have upgraded the Sequel instrument in 2021, which enabled a significant increase of the HiFi long read production.

During 2021, we carried out 53 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, single-cell trnascriptome and ChIP-seq.

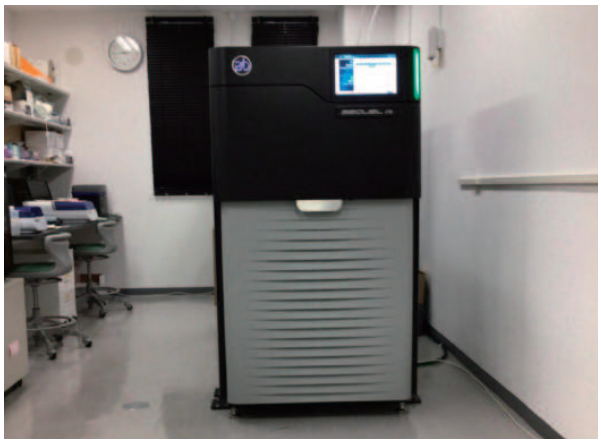


Figure 1. Next-generation sequencer

Proteomics

As is listed below, two types of mass spectrometers and two protein sequencers are used for proteome studies in our facility. In 2021, we analyzed approximately 600 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)

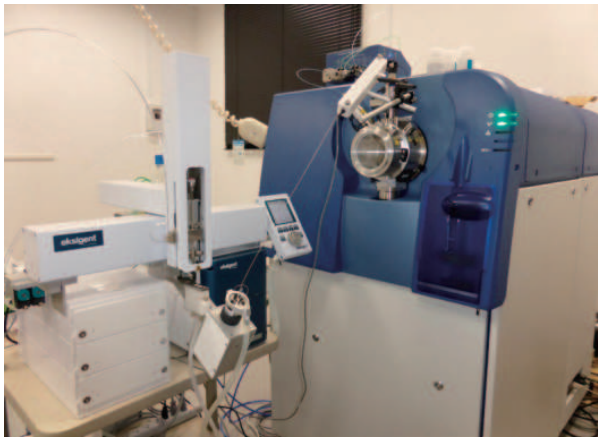


Figure 2. LC-MS/MS system

Other analytical instruments (excerpts)

- Cell sorter (SONY SH800)
- Bioimaging analyzer (BIO-RAD ChemiDoc XRS+ ; Fujifilm LAS 3000 mini; GE FLA9000)
- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500, QuantStudio 3)
- Ultracentrifuge (Beckman XL-80XP etc.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1, 10x Genomics Chromium X)

Publication List on Cooperation:

〔Original papers〕

- Goto, T., Soyano, T., Liu, M., Mori, T., and Kawaguchi, M. (2022). Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus*. Proc. Natl. Acad. Sci. U.S.A. *119*. e2116549119. DOI: 10.1073/pnas.2116549119
- Hasegawa, Y., Ueno, S., Wei, F.-J., Matsumoto, A., Uchiyama, K., Ujino-Ihara, T., Hakamata, T., Fujino, T., Kasahara, M., Bino Takahiro and Yamaguchi, K., Shigenobu, S., Tsumura, Y., and Moriguchi, Y. (2021). Identification and genetic diversity analysis of a male-sterile gene (MS1) in Japanese cedar (Cryptomeria japonica D. Don). Sci. Rep. *11*, 1496. DOI: 10.1038/s41598-020-80688-1
- Igawa-Ueda, K., Ikuta, T., Tame, A., Yamaguchi, K., Shigenobu, S., Hongo, Y., Takaki, Y., Fujikura, K., Maruyama, T., and Yoshida, T. (2021). Symbiont Transmission onto the Cell Surface of Early Oocytes in the Deep-sea Clam *Phreagena okutanii*. Zoolog. Sci. *38*, 140–147. DOI: 10.2108/zs200129
- Ishida, S., Suzuki, H., Iwaki, A., Kawamura, S., Yamaoka, S., Kojima, M., Takebayashi, Y., Yamaguchi, K., Shigenobu, S., Sakakibara, H., Kohchi, T., and Nishihama, R. (2022). Diminished Auxin Signaling Triggers Cellular Reprogramming by Inducing a Regeneration Factor in the Liverwort Marchantia polymorpha. Plant Cell Physiol. *63*, 384–400. DOI: 10.1093/pcp/pcac004
- Ishishita, S., Kitahara, S., Takahashi, M., Iwasaki, S., Tatsumoto, S., Hara, I., Kaneko, Y., Kinoshita, K., Yamaguchi, K., Harada, A., Ohmori, Y., Ohkawa, Y., Go, Y., Shigenobu, S., Matsuda, Y., and Suzuki, T. (2022). Uterus-specific transcriptional regulation underlies eggshell pigment production in Japanese quail. PLoS One *17*, e0265008. DOI: 10.1371/journal.pone.0265008
- Korgaonkar, A., Han, C., Lemire, A.L., Siwanowicz, I., Bennouna, D., Kopec, R.E., Andolfatto, P., Shigenobu, S., and Stern, D.L. (2021). A novel family of secreted insect proteins linked to plant gall development. Curr. Biol. *31*, 1836-1849.e12. DOI: 10.1016/j.cub.2021.01.104
- Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., and Shigenobu, S. (2021). Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*. eLife *10*, e60176. DOI: 10.7554/eLife.60176
- Miyazaki, S., Fujiwara, K., Kai, K., Masuoka, Y., Gotoh, H., Niimi, T., Hayashi, Y., Shigenobu, S., and Maekawa, K. (2021). Evolutionary transition of doublesex regulation from sex-specific splicing to male-specific transcription in termites. Sci. Rep. *11*, 15992. DOI: 10.1038/s41598-021-95423-7
- Okamoto, S., Kawasaki, A., Makino, Y., Ishida, T., and Sawa, S. (2022). Long-distance translocation of CLAVATA3/ESR-related 2 peptide and its positive effect on roots sucrose status. Plant Physiol. *189*, 2357–2367. DOI: 10.1093/plphys/kiac227
- Okamoto, S., Kawasaki, A., and Makino, Y. (2022). Characterization of Oligopeptides in *Solanum lycopersicum* Xylem Exudates. Life *12*. 592. DOI: 10.3390/life12040592
- Sato, K., Uehara, T., Holbein, J., Sasaki-Sekimoto, Y., Gan, P., Bino, T., Yamaguchi, K., Ichihashi, Y., Maki, N., Shigenobu, S., Ohta, H., Franke, R.B., Siddique, S., Grundler, F.M.W., Suzuki, T., Kadota, Y., and Shirasu, K. (2021). Transcriptomic Analysis of Resistant and Susceptible Responses in a New Model Root-Knot Nematode Infection System Using *Solanum torvum* and *Meloidogyne arenaria*. Front. Plant Sci. *12*, 680151. DOI: 10.3389/fpls.2021.680151
- Shigenobu, S., Hayashi, Y., Watanabe, D., Tokuda, G., Hojo, M.Y., Toga, K., Saiki, R., Yaguchi, H., Masuoka, Y., Suzuki, R., Suzuki, S., Kimura, M., Matsunami, M., Sugime, Y., Oguchi, K., Niimi, T., Gotoh, H., Hojo, M.K., Miyazaki, S., Toyoda, A., Miura, T., and Maekawa, K. (2022). Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: Gene duplication facilitates social evolution. Proc. Natl. Acad. Sci. U.S.A. *119*, e2110361119. DOI: 10.1073/pnas.2110361119
- Subhankar, B., Yamaguchi, K., Shigenobu, S., and Aoki, K. (2021). *Trans*-species small RNAs move long distances in a parasitic plant complex. Plant Biotechnol. *38*, 187–196. DOI: 10.5511/plantbiotechnology.21.0121a
- Takeuchi, T., Matsubara, H., Minamitani, F., Satoh, Y., Tozawa, S., Moriyama, T., Maruyama, K., Suzuki, K.T., Shigenobu, S., Inoue, T., Tamura, K., Agata, K., and Hayashi, T. (2022). Newt Hoxa13 has an essential and predominant role in digit formation during development and regeneration. Development *149*, dev200282. DOI: 10.1242/dev.200282
- Tominaga, T., Miura, C., Sumigawa, Y., Hirose, Y., Yamaguchi, K., Shigenobu, S., Mine, A., and Kaminaka, H. (2021). Conservation and Diversity in Gibberellin-Mediated Transcriptional Responses Among Host Plants Forming Distinct Arbuscular Mycorrhizal Morphotypes. Front. Plant Sci. *12*, 795695. DOI: 10.3389/fpls.2021.795695
- Yoshida, Y., Shaikhutdinov, N., Kozlova, O., Itoh, M., Tagami, M., Murata, M., Nishiyori-Sueki, H., Kojima-Ishiyama, M., Noma, S., Cherkasov, A., Gazizova, G., Nasibullina, A., Deviatiiarov, R., Shagimardanova, E., Ryabova, A., Yamaguchi, K., Bino, T., Shigenobu, S., Tokumoto, S., Miyata, Y., Cornette, R., Yamada, T.G., Funahashi, A., Tomita, M., Gusev, O., and Kikawada, T. (2022). High quality genome assembly of the anhydrobiotic midge provides insights on a single chromosome-based emergence of extreme desiccation tolerance. NAR Genomics Bioinforma. *4*, lqac029. DOI: 10.1093/nargab/lqac029

〔Review article〕

- Miura, T., Oguchi, K., Yamaguchi, H., Nakamura, M., Sato, D., Kobayashi, K., Kutsukake, N., Miura, K., Hayashi, Y., Hojo, M., Maekawa, K., Shigenobu, S., Kano, T., and Ishiguro, A. (2022). Understanding of superorganisms: collective behavior, differentiation and social organization. Artif. Life Robot. *27*, 204–212. DOI: 10.1007/s10015-022-00754-x

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

Technical Staff:	*KONDO, Maki*
	TANIGUCHI-SAIDA, Misako
Visiting Undergraduate:	*YOSHIKAWA, Ryusei*
Technical Assistant:	*ICHIKAWA, Chiaki*
	ASAO, Momoko
	NAKAGAWA, Mami
	AOYAMA, Chie

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 great 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 both biological and artificial organic molecules have been conducted since it has been 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.

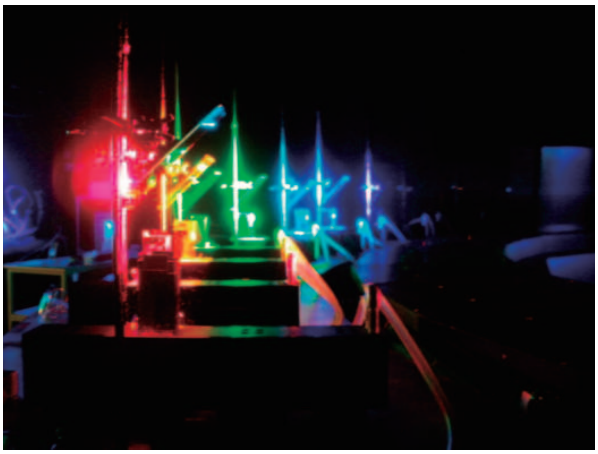


Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously onto samples stored in cooling chambers.

Microscopes

This facility also provides bioimaging machinery (Figure 2), 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 have 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.

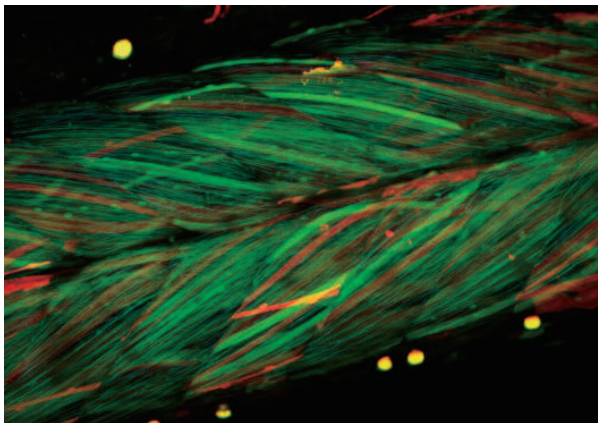


Figure 2. Multi-color confocal microscope image of muscle in a transgenic medaka larva. Each muscle cell expressed randomly various color variants of fluorescent protein, such as Cerulean, GFP, YFP and dsRed. The transgenic line was provided by NBRP Medaka.

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). Subsequently, Dr. Nonaka has conducted and supported roughly 10 Collaborative Research Program projects for Integrative Bioimaging. The IR-LEGO, developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST), can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser. This microscope technology can be applied to thermal biology through local heating (the details of this are provided in the next section). IR-LEGO was also used for about 10 Collaborative Research projects, including applications aimed at both animals and plants.

Workshop, Symposium and Training course

In 2021, we held the 9th biological image processing training course in cooperation with Drs. Kagayaki Kato, Shigenori Nonaka, Yasuhiro Kamei, Takashi Murata and Hiroshi Koyama. The course was held in an online meeting format due to the COVID-19 pandemic. We have also started a new course "Optical Microscopy Principle Training Course" cooperation with Drs. Joe Sakamoto, Yasuhiro Kamei, Atsushi Taniguchi, Shigenori Nonaka in NIBB and Motosuke Tsutsumi and Kohei Otomo in ExCELLS. In this course, participants learned microscopy principles thorough lectures and built bright-field and fluorescent microscopes by themselves using optical devices, such as lenses, filters, light sources and cameras (Figure 3). We additionally hold some training courses and seminars related to microscopy and image analysis, including their technologies and applications.

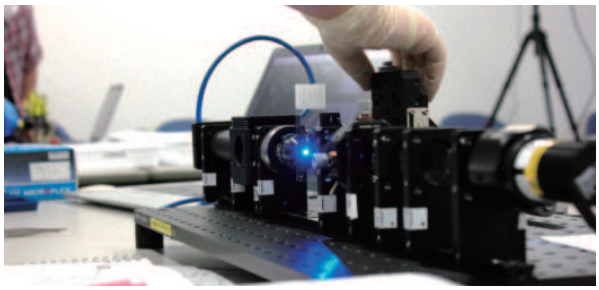


Figure 3. A scene from the 1st Optical Microscopy Principle Training Course. The participants built up a basic microscope by themselves using optical devices.

Publication List on Cooperation

〔Original papers (Selected)〕

- Ansai, S., Mochida, K., Fujimoto, S., Mokodongan, D.F., Sumarto, B.K.A., Masengi, K.W.A., Hadiaty, R.K., Nagano, A.J., Toyoda, A., Naruse, K., Yamahira, K., and Kitano, J. (2021). Genome editing reveals fitness effects of a gene for sexual dichromatism in Sulawesian fishes. Nat. Commun. *12*. 1350. DOI: 10.1038/s41467-021-21697-0
- Beppu, K., Tsutsumi, R., Ansai, S., Ochiai, N., Terakawa, M., Mori, M., Kuroda, M., Horikawa, K., Tomoi, T., Sakamoto, J., Kamei, Y., Naruse, K., and Sakaue, H. (2022). Development of a screening system for agents that modulate taste receptor expression with the CRISPR-Cas9 system in medaka. Biochem. Biophys. Res. Commun. *601*, 65–72. DOI: 10.1016/j.bbrc.2022.02.082
- Goto, T., Soyano, T., Liu, M., Mori, T., and Kawaguchi, M. (2022). Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in Lotus japonicus. Proc. Natl. Acad. Sci. U.S.A. *119*, e2116549119. DOI: 10.1073/pnas.2116549119
- Kaneko, E., Sato, H., and Fukamachi, S. (2021). Validation of the three-chamber strategy for studying mate choice in medaka. PLoS One *16*, e0259741. DOI: 10.1371/journal.pone.0259741
- Kinoshita, N., Yamamoto, T.S., Yasue, N., Takagi, C., Fujimori, T., and Ueno, N. (2022). Article Force-dependent remodeling of cytoplasmic ZO-1 condensates contributes to cell-cell adhesion through enhancing tight junctions. iSCIENCE *25*, 103846. DOI: 10.1016/j.isci.2022.103846
- Matsuo, M., Kamei, Y., and Fukamachi, S. (2021). Behavioural red-light sensitivity in fish according to the optomotor response. R. Soc. Open Sci. *8*, 210415. DOI: 10.1098/rsos.210415
- Mohri, T., and Kyozuka, K. (2022). Starfish oocytes of A. pectinifera reveal marked differences in sperm-induced electrical and intracellular calcium changes during oocyte maturation and at fertilization. Mol. Reprod. Dev. *89*, 3–22. DOI: 10.1002/mrd.23544
- Tokanai, K., Kamei, Y., and Minokawa, T. (2021). An easy and rapid staining method for confocal microscopic observation and reconstruction of three-dimensional images of echinoderm larvae and juveniles. Dev. Growth Differ. *63*, 478–487. DOI: 10.1111/dgd.12758
- Wu, D., Arakawa, H., Fujita, A., Hashimoto, H., Hibi, M., Naruse, K., Kamei, Y., Sato, C., and Kitajima, K. (2021). A point-mutation in the C-domain of CMP-sialic acid synthetase leads to lethality of medaka due to protein insolubility. Sci. Rep. *11*, 23211. DOI: 10.1038/s41598-021-01715-3
- Zeng, C.-W., Kamei, Y., Shigenobu, S., Sheu, J.-C., and Tsai, H.-J. (2021). Injury-induced Cavl-expressing cells at lesion rostral side play major roles in spinal cord regeneration. Open Biol. *11*, 200304. DOI: 10.1098/rsob.200304

〔Research activity by Yasuhiro Kamei〕

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

DATA INTEGRATION AND ANALYSIS FACILITY

Associate Professor
UCHIYAMA, Ikuo

Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
SUGIURA, Hiroki
Technical Assistant: *KOTANI, Keiko*

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), DB-HABs (harmful algal blooms), and ChaetoBase (*Chaetoceros gracilis*).

Figure 1. Biological Information Analysis System

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 (https://www.nibb.ac.jp/en).

Research activity by Ikuo Uchiyama

Associate 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
	KITAZUMI, Noriaki

The 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.

Figure 1. Mouse breeding room in the Yamate area

Figure 2. Breeding cages for replacement in the clean corridor

From April 1, 2021 to March 31, 2022, 3,388 mice (1 transgenic line and wild-type) were brought into the facility in the Yamate area, and 30,944 mice (including pups bred in the facility) were taken out.

A number of strains of genetically altered mice from outside the facility were brought into this area after microbiological cleaning using *in vitro* fertilization-embryo transfer techniques (1 transgenic line), and stored using cryopreservation (34 transgenic lines). The frozen eggs of 43 mice lines were taken out of the facility.

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

Figure 3. Large sized autoclave in the Yamate area

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 37.5 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.

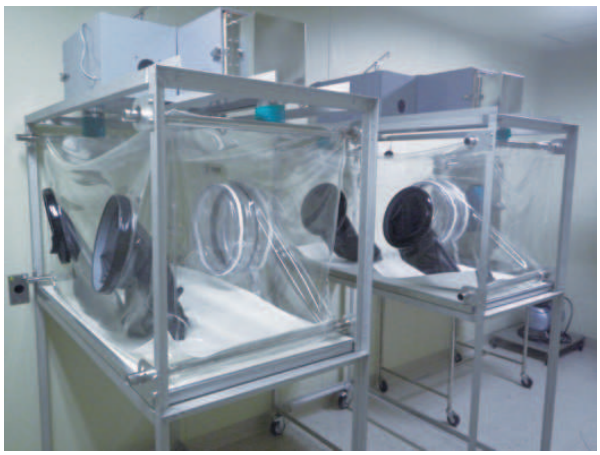


Figure 4. Vinyl isolators in the quarantine room

In 2021, 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 general cultivation of plants and the rearing of several animal species that do not qualify for housing in other facilities.

The Plant Culture Laboratory equips and manages approximately 75 culture boxes or growth chambers and 13 rooms with the P1P physical containment level required for established and emerging model plants, such as thale cress (*Arabidopsis thaliana*), rice (*Oryza sativa*), moss (*Physcomitrella patens*), liverwort (*Marchantia polymorpha*), green alga (*Chlamydomonas reinhardtii*), and several other flowering plants including carnivorous plants. The facilities are also used to grow the sea anemone *Exaiptasia pallida*. The culture space is used throughout the year by >70 researchers from external and internal groups.

In addition to regular culture conditions, extreme environmental conditions in terms of light and temperature are available for experiments. Three chambers (3.4 m^2 each) with controllable CO_2, humidity, temperature, and light (max 70,000 lux) conditions are also available. A tissue culture rack with dimmable LEDs and pulse-width modulation con-

trollers is available for algae cultures that must be exposed to precise light concentrations. Autotrophic and heterotrophic culture devices are also available for researchers working with cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches and a safety cabinet. Various analytical instruments, including three flow cytometry systems and a DUAL-PAM system to measure DNA content and chlorophyll fluorescence, respectively, are also available. A liquid handling system for fully automated and simultaneous *in situ* hybridization of sections of up to 60 glass slides is also provided.

There is also a 386 m^2 experimental farm next to the NIBB Myodaiji area building for maintaining Japanese morning glories and related *Ipomoea* species, several carnivorous plants, castor beans, and other flowering plants that must be cultivated outdoors. Three heated greenhouses (measuring 44, 44, and 45 m^2) contain sensitive carnivorous plants and the periodically mass-flowering plant *Strobilanthes flexicaulis*. Four air-conditioned greenhouses (4, 6, 9, and 9 m^2) are provided for the cultivation of Japanese morning glories and additional carnivorous plants. Two of the air-conditioned greenhouses (9 and 18 m^2) meet the P1P physical containment level and are available for experiments using transgenic rice plants, Japanese morning glories, and carnivorous 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 Japanese rhinoceros beetles and the common grass yellow butterfly.

Between April 2021 and March 2022, a ploidy analyzer was introduced. It is a compact flow cytometer that is suitable for ploidy and genome size analyses of plants, animals, and microorganisms. The liquid handling system has been updated to support whole-mount *in situ* hybridization.

There are two groups in the Laboratory of Biological Diversity, and the assistant professors at this facility, Dr. A. Hoshino and Dr. K. Tsugane, are the principal investigators of each group. For additional details, please refer to the laboratory's webpage.

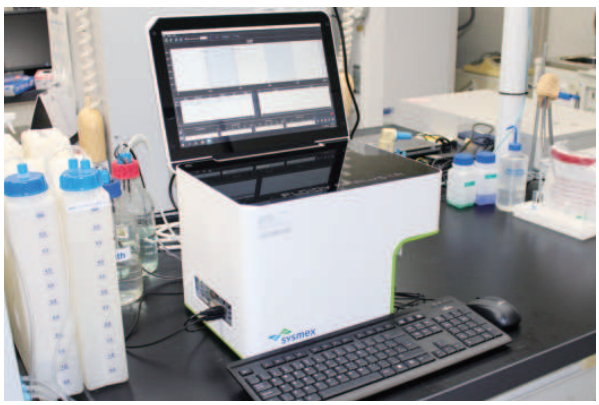


Figure 5. Newly introduced ploidy analyzer.

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/P3 physical containment level, and is routinely used for DNA recombination experiments.

Figure 6. Equipment for P3A experiments

National Bioresource Project

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

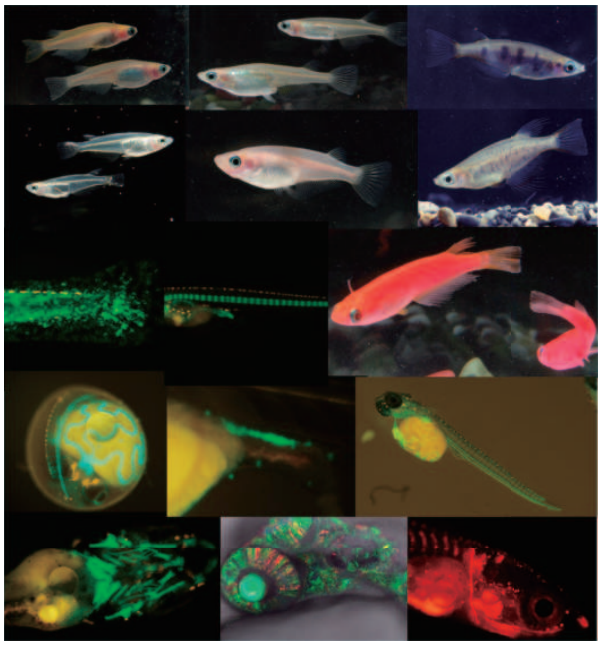
I. NBRP Medaka (*Oryzias latipes*)

Project Manager: NARUSE, Kiyoshi

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 a genome editing platform using CRISPR/Cas9. Using collaborative research support, researchers can visit NIBB to generate mutants by genome editing.

With the approval of the second supplementary budget for FY2020, we were able to install a cabinet-type fish tank washing machine. This has freed up our technical support staff from the need to wash the tanks by hand and has allowed us to focus more on breeding and management, which requires more human work. In addition, a system to remotely monitor the temperature, humidity, and illumination in the medaka breeding rooms and the water temperature in the breeding tanks was installed. The air conditioner in the breeding room was also upgraded. We have continuously monitored the medaka breeding conditions using these systems. In the collection of individual resources, we collected 26 strains (achievement rate 173%). As a result, the entire core institute now has 730 strains (achievement rate 125%). A total of 411 strains (achievement rate 117%) were provided. 76 DNA clones were provided (achievement rate 38 %). As of FY2021, 395 hatching enzyme tubes (achievement rate 197%) were provided; thus the numerical targets for collection, conservation, and provisioning were met for the entire project, except for the number of clones provided. To enhance online contents, “The Life of My Master Dr. Tatsuo Aida” (edited by Tetsuro Takeuchi), “Biological Experiments for Elementary, Middle, and High School Students” (written by Kunisuke Takeuchi), and “Medaka Experiments Anyone Can Do” (written by Kunisuke Takeuchi) were released from NBRP Medaka in April 2021. In October 2021, a new database, “3D atlas and single cell transcriptome data of medaka pituitary” was made available on NBRP Medaka. On September 16-17, 2021, we participated in the 27th Small Fish Research Meeting, as well as the Small Fish Community Meeting. The 44th Annual Meeting of the Molecular Biology Society of Japan (December 1-3, 2021, Pacifico Yokohama) was held onsite, and we promoted the project with Utsunomiya University at a booth at the “National BioResource Project (NBRP) - A lineup of bioresources.



Medaka resources provided from NBRP medaka

II. NBRP Morning Glory (*Ipomoea nil*)

Project Manager: HOSHINO, Atsushi

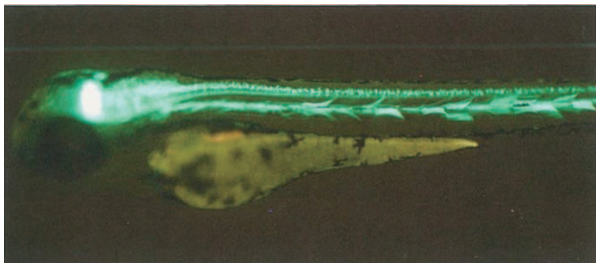
Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan. Studied worldwide, it is commonly investigated in the fields of plant physiology and genetics. NIBB collects, develops, and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning Glory in collaboration with the core organization center at Kyushu University. From April 2021 to March 2022, we collected 10 mutant lines and 6 transgenic lines. Additionally, we provided 10 mutant lines, 2 transgenic lines, and 13 DNA clones to both local and international biologists. Finally, we analyzed whole-genome sequences of 100 mutant lines using next-generation sequencers to develop genetic variation databases.

Left: The genome database (http://viewer.shigen.info/asagao/) containing the whole-genome sequence, transcriptome sequences, and end sequences of the EST and BAC clones. Mutation sequence information links the genome and mutant databases. Right: A mutant flower phenotype

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 observation. 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 of the Interuniversity Bio-Backup Project (IBBP Center)

Head and Specially Appointed Professor
NARUSE, Kiyoshi

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

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

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

Figure 1. IBBP Center.

The IBBP Center includes:

- earthquake proof structures capable of withstanding even very large-scale quakes which are equipped with emergency backup power generators,
- cryopreservation facilities equipped with automatic liquid nitrogen feeding systems,
- deep freezers, and refrigerated storage (mainly for seed stocks),

as well as all manner of automated laboratory equipment, cell culture tools, and the latest equipment necessary to back up the genetic resources in a collaborative manner. The specific preservation methods used are the freezing of animal sperm and eggs, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

When university satellite hubs receive preservation requests involving biological resources from researchers, they report to the Managing Project Committee of IBBP (which is comprised of NIBB faculty members and other satellite institutes), where the relevance of the request is reviewed. If the request is approved, the biological resources that are to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated), and their particulars registered into a database. In the event of a disaster leading to the loss of a researcher's own biological resources, preserved samples will be promptly returned to the researcher so they can quickly resume their work.

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

I. Current status of back up available for biological resources

In 2021, the IBBP Center stored 5,264 384-well and 112 96-well plates consisting of as cDNA/BAC clones, 17,669 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 8,498 133mm-straw tubes for sperm and 671 seed samples. In total 2,049,024 samples were stored.

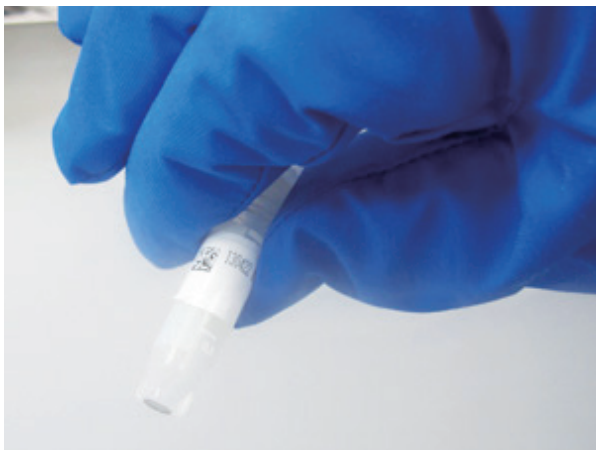


Figure 3. Cryo tube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

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

As the IBBP Center can only accept biological resources which can be cryopreserved in liquid nitrogen, researchers cannot backup biological resources for which cryopreservation methods are not well established. In order to increase the usability of IBBP, we started a collaborative research project for the development of new long-term storage technologies and cryo-biological study in 2013. This collaborative research focuses on two goals: 1) The establishment of new storage technologies for biological resources for which long-term storage is unavailable. 2) Basic cryobiological research enabling us to improve low temperature storage for biological resources. In 2021, we have conducted 10 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 2021 on November 11-12, 2021 online, because of the COVID-19 pandemic. We had 227 participants from several fields covering physics, chemistry, biology, and technology. As the special lectures, We had 4 special lectures, the current status of the preservation of cultured cells and germ cells of endangered species at the National Institute for Environmental Studies, the relationship between protein conformational changes and additive properties that occur during freezing and drying of aqueous solutions of lyophilized pharmaceuticals, the analysis of amino acid and gene composition, elucidation of biochemical and physicochemical properties, structure-function relationship analysis, and development of mass production technology for several antifreeze proteins from Japanese plants and animals and the fundamentals of successful cryopreservation as well as recent preservation methods and facilities.

Figure 4. Group photo of Cryopreservation conference 2021

CENTER FOR THE DEVELOPMENT OF NEW MODEL ORGANISMS

Head
UENO, Naoto

Professor
SHIGENOBU, Shuji

Specially Appointed Associate Professor
SUZUKI, Ken-ichi

Postdoctoral Fellow:	*KISHIMOTO, Mariko*
	SHIBATA, Yuki
Technical Assistant:	*TAKAYAMA, Ayuko*
	SANBO, Chiaki

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.

SUZUKI Group

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

Postdoctoral Fellow:	*SHIBATA, Yuki*
Technical Assistant:	*TAKAYAMA, Ayuko*
	SANBO, Chiaki

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.

1-1 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.

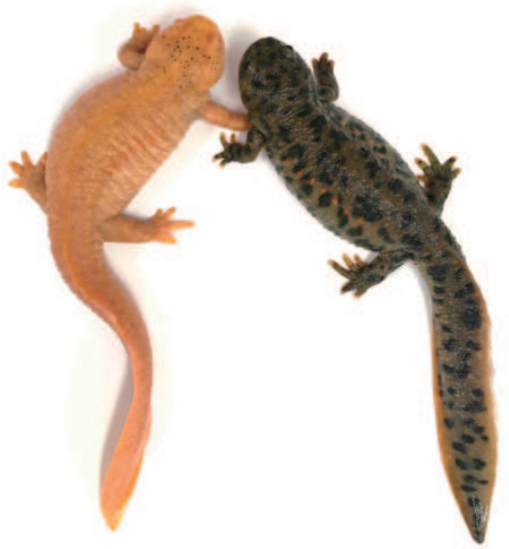


Figure 1. *tyrosinase* crispant in *P. waltl*. A knock-out founder of tyrosinase, a melanin synthesis enzyme, and wild newt (left and right, respectively). *tyr* crispant shows full albinism.

1-2 Finding new model organisms and deciphering organ regeneration

One of our missions is to discover unique organisms and develop them as new model organisms for basic biology. A recent example of this is our recent establishment of the newt *Pleurodeles waltl* as an experimental model animal for regenerative biology using NGS and genome editing techniques. *P. waltl* possesses several excellent characteristics as a model animal: easy breeding, short sexual maturation period, remarkable regenerative capacity and comparatively high efficiency of genome editing (Figure 2). We are currently 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.

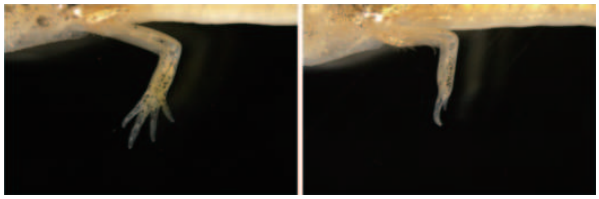


Figure 2. A limb-specific enhancer (ZRS/MFCS1) of *sonic hedgehog* crispant in *P. waltl*. Phenotypes of limb regeneration in wild and ZRS/MFCS1 crispant (left and right, respectively). Unlike in normal limb regeneration in the wild type, severe reduction of digit formation was seen in ZRS/MFCS1 crispant.

Publication List:

〔Original papers〕

● Takeuchi, T., Matsubara, H., Minamitani, F., Satoh, Y., Tozawa, S., Moriyama, T., Maruyama, K., Suzuki, K.T., Shigenobu, S., Inoue, T., Tamura, K., Agata, K., and Hayashi, T. (2022). Newt Hoxa13 has an essential and predominant role in digit formation during development and regeneration. Development *149*, dev200282. DOI: 10.1242/dev.200282

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Technical Staff:	*MATSUDA, Yoshimi*
	(Radiation Protection Supervisor, Myodaiji area)
	SAWADA, Kaoru
	IINUMA, Hideko
Technical Assistant:	*HAYASHI, Tomoko*

The Center for Radioisotope Facilities (CRF) provides a well-established and comfortable environment for natural science researchers across Japan as part of the Inter-University Research Institute Corporation. Center staff maintain controlled areas in compliance with relevant laws and monitor the purchase and transfer of radioisotopes (RIs). The following is an outline of the major activities that CRF conducted in 2021.

1. Revision of the CRF's rules

The CRF's rules were revised twice in 2021. In April 2021, to respond to the elimination of the seal and signature and the reorganization of the IMS technical organization, the CRF's two rules, the Center for Radioisotope Facilities Myoudaiji District Experimental Facilities Radiation Damage Protection Rules and the Center for Radioisotope Facilities Myoudaiji District Experimental Facilities Radiology Worker Registration Procedure Guide, were revised accordingly. In July 2021, to meet the revision of the Japanese relevant laws, the CRF's rule (Center for Radioisotope Facilities Myoudaiji District Experimental Facilities Radiation Damage Protection Rules) was revised. The main changes include:

1) the measurement of exposure doses in the lens of the eyes (from "150mSv per year" to "50mSv per year and 100mSv per 5 years")
2) the method of recording radiation dose measurements and calculations

2. Renewal of the RI handling management system

The relevant laws require the handling (acceptance, use, dispose and dispensing) of RIs to be recorded. The CRF has used a PC software (the RI handling management system, Aloka ISR1000), but the software is no longer compatible with the current situation. With the approval of the CRF Steering Committee, we renewed the software in March 2022 using budget allocated from NIBB and NIPS.

3. Cleaning and checking the radioactive wastewater storage tanks

All wastewater from the CRF is stored in the radioactive wastewater storage tanks. Wastewater is discharged after confirming that the concentration of RIs in the water is below the legal concentration limit. In February 2022, we commissioned contractors to clean and inspect the inside of the tanks. By this checking, the wastewater storage tanks were diagnosed to continue to be usable.

The figures for training courses of RI handling are shown in Table 1. The annual changes of the CRF registrants and the total number of entrances to the CRF per fiscal year are shown in Figure 1. The numbers of the CRF registrants and users from April 2021 to March 2022 are shown in Table 2. During this period the total number of the users and visitors counted by the access control system was 802 (Table 3). The balance of RIs received and used at the CRF is shown in Table 4.

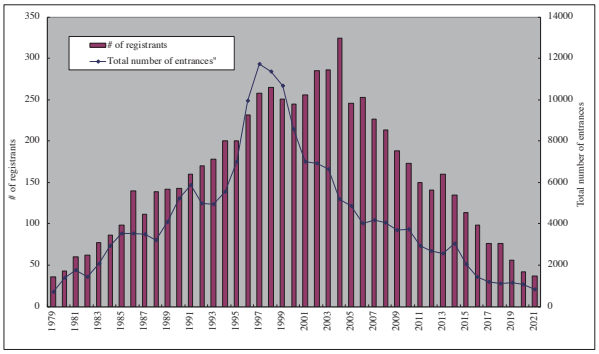


Figure 1. Annual number of the CRF registrants and the total number of entrances to the CRF

Table 1. Training courses offered for radiation workers during fiscal year 2021.

Training course	# of participants
Introductory course for beginners	0
Introductory course for experts	5
Users training course	32
Radioisotope handling practice	1

Table 2. Number of the CRF registrants and users during fiscal year 2021.

Type	The number of persons
# of registrants	37
# of users	16

Table 3. Total number of uses by individual users and visitors during fiscal year 2021.

Type	The number of persons
# of users	688
# of visitors	114

Table 4. RIs received and used (in kBq) during fiscal year 2021.

Nuclide	Situation	Radioactivity
^{32}P	Received	59,200
^{32}P	Used	46,450
^{14}C	Received	18,500
^{14}C	Used	23,865
^{3}H	Received	0
^{3}H	Used	37

RESEARCH ENHANCEMENT STRATEGY OFFICE


Director
HASEBE, Mitsuyasu


Vice-Director
YOSHIDA, Shosei


Vice-Director
MANO, Shoji

The Research Enhancement Strategy Office (RESO) was established in 2013 to manage the NIBB action plans proposed by the Director and the Advisory Committee for Programming and Management as an Inter-University Research Institute: (1) promoting internationally top-notch research and (2) providing opportunities for cutting-edge researchers throughout Japan to gather and engage in activities aimed at exploring future academic fields and creating new principles. RESO is composed of six groups with the following missions: (1) planning and evaluation, (2) joint utilization of NIBB facilities and joint research between NIBB members and researchers throughout the world, (3) domestic and international cooperation, (4) promotion of the diversity and education of young researchers, (5) dissemination of information and enhancement of public relations, and (6) collaboration between academia and industry. RESO is mainly managed by University Research Administrators who hold PhDs and have experience in both research and management. RESO cooperates with the mother institute NINS's Research Enhancement Promotion Headquarters. The activities of this office are partly supported by the Program for Promoting the Enhancement of Research Universities of the Ministry of Education, Culture, Sports, Science and Technology.

PLANNING AND EVALUATION GROUP

Professor: *HASEBE, Mitsuyasu*
KAWAGUCHI, Masayoshi
Specially Appointed Assistant Professor (URA):
KURATA, Tomoko
TATEMATSU, Kiyoshi
Assistant Professor: *JOHZUKA, Katsuki*
FUJITA, Hironori

This group compiles reports describing NIBB activities for evaluation and budget proposals.

The group's main activities in 2021 were as follows:

1) Prepared reports on 2020 NIBB activities for MEXT.

2) Prepared reports for 3rd medium-term objectives for the evaluation of education and research.

3) Prepared application forms for the MEXT FY2022 budget demands.

4) Prepared reports for the NINS auditors.

5) Conducted follow-up investigation of the activity of the Research University Strengthening Promotion project.

6) Summarized 2020 and 2021 NIBB activities for the annual external reviewer meeting on 8 March 2022, managed the meeting, and prepared the report.

7) Prepared the proposal on mid-term objectives and plans from 2022 to 2027.

8) Published the Annual Report 2020.

PUBLIC RELATIONS GROUP

Professor: *FUJIMORI, Toshihiko*
Specially Appointed Assistant Professor (URA):
KURATA, Tomoko
Technical Assistants: *BAN, Misato*
HOSHINO, Maki
UCHIMURA, Ai

This group actively facilitates communication between NIBB and the public, school teachers, and scientific research communities and describes the activities of NIBB to a wider audience.

The group's main activities in 2021 were as follows:

1) Press releases

The group issued press releases concerning NIBB's scientific achievements to newspaper and magazine reporters via leaflets, e-mails and web pages. We also arranged press conferences.

2) Updated and maintained the NIBB web page

3) Edited publications, produced posters and leaflets

The group published "NIBB News" (an intra-institutional newsletter in Japanese) and brochures introducing NIBB. We also designed and distributed posters about NIBB events.

4) Managed social media

The group operated Twitter and Facebook accounts to communicate the activities of NIBB to the public. We also created videos introducing NIBB's research that are posted on YouTube.

5) Public relations activities to recruit graduate students

The group organized four graduate school information sessions for prospective students during the year.

6) Organized scientific outreach programs

The group organized online outreach events for the public and coordinated science classes for middle and high school

Liaison and Cooperation Group

Professor:	*UENO, Naoto*
	NARUSE, Kiyoshi
	MORITA, Miyo T.
Specially Appointed Assistant Professor (URA):	
	TATEMATSU, Kiyoshi
Technical Staff:	*AKIMOTO-KATO, Ai*
Research Staff:	*TANAKA, Ayako*
Technical Assistant:	*TAKAHASHI, Ritsue*
	COWAN, Glen

This group supports and coordinates NIBB's activities related to research collaborations and cooperation with universities and research institutes in Japan and abroad.

The main activities performed by this group from April 2021 to March 2022 were as follows:

1) Coordinated the following conferences/symposia:

- The 2nd meeting for NIBB-Center for Organismal Studies (COS) Heidelberg International Collaborations Lecture series #2 "Stem Cell". Zoom online, June 25, 2021 (p. 112)

- NIBB-Institute of Advanced Medical Sciences (IAMS), Tokushima University, joint seminar "Toward Strengthening Collaboration and Cooperation". Zoom online, October 22, 2021 (p. 113)

- Cryopreservation Conference 2021. Zoom online, November 11–12, 2021 (p. 104)

- The 3rd Chubu University-NIPS-NIBB joint seminar "AI to Life System". Okazaki Conference Center together with Zoom online (Hybrid), Okazaki, Japan. March 28, 2022 (p. 114)

- The 3rd meeting for NIBB-COS Heidelberg International Collaborations Lecture series #3 "Cell Signaling / Cell Biology". Zoom online, March 31, 2021 (p. 112)

2) Supported the acceptance of researchers to NIBB from abroad related to the following events:

- Family of a specially appointed assistant professor from Poland (September 2021)

3) Supported the following education-related programs:

- NIBB Internship Program 2021 (p. 118)

Collaborative Research Group

Professor:	*TAKADA, Shinji*
	SHIGENOBU, Shuji
Specially Appointed Associate Professor (URA):	
	KAMEI, Yasuhiro
Associate Professor:	*UCHIYAMA, Ikuo*
Specially Appointed Assistant Professor:	
	KURATA,Tomoko
	TATEMATSU,Kiyoshi
Technical Assistant:	*ICHIKAWA, Mariko*
	ICHIKAWA, Chiaki

This group acts as a hub to promote collaborative projects among multiple researchers through the exchange of information and the development of new equipment and methods.

The main activities of this group in 2021 were as follows:

1) Hosted collaborative projects.

2) Operated and managed NOUS (NINS Operation Use System), an online platform to manage collaborative projects.

3) Organized the Genome Informatics Training Course (GITC) and the BioImage Analysis Training Course (BIATC).

4) Made public announcements of the Cooperative and Collaborative Research System in NIBB to the basic biology research communities at research meetings and conferences.

Young Researcher Support Group

Professor:	*NIIMI, Teruyuki*
Assistant Professor:	*KOMINE, Yuriko*

NIBB provides PhD courses for graduate students while acting as Department of Basic Biology, School of Life Science, The Graduate University for Advanced Studies, SOKENDAI (SOKENDAI is the commonly used name for the university). The Young Researcher Support Group provides support for graduate students (SOKENDAI students and special research students from other universities).

The group's main activities in 2021 were as follows:

1) Coordinated and managed lectures and other activities, including periodical research presentations by students.

2) Organized NIBB's programs related to PhD courses, such as the orientation program for new students and Open Campus Day online for prospective students.

3) Cooperated to organize interdepartmental programs in SOKENDAI such as the Life Science Retreat.

4) Gathered and provided useful information for both students and faculty members.

COLLABORATIVE INNOVATION GROUP

Professor: *MINAGAWA, Jun*
Specially Appointed Associate Professor: *KANAI, Masatake*
Professor: *MINAGAWA,Jun*
Associate Professor: *KODAMA, Ryuji*
Specially Appointed Assistant Professor: *KANAI, Masatake*

This group is responsible for activities that bridge the gap between NIBB researchers and the business community.

The group's main activities in 2021 were as follows:

1) Supported the application for JST's A-step, a research grant for practical use.

2) Supported participation in Innovation Japan, a trade fair for university seeds.

3) Held a seminar on the support system for industry-university collaboration.

4) Negotiated a license agreement with a company.

5) Provided support for joint research agreements between NIBB researchers and companies.

TECHNICAL DIVISION

Head
MIWA, Tomoki

Common Facility Group

Chief: *MORI, Tomoko*

● ***NIBB Core Research Facilities***
Unit Chief: *KONDO, Maki*
Vice-Unit Chief: *MAKINO, Yumiko*
YAMAGUCHI, Katsushi
NISHIDE, Hiroyo
Technical Staff: *NAKAMURA, Takanori*
TANIGUCHI-SAIDA, Misako
SUGIURA, Hiroki
MORI, Shogo
Technical Assistant: *ICHIKAWA, Chiaki*
ICHIKAWA, Mariko
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

● ***IBBP***
Technical Staff: *AKIMOTO-KATO, Ai*

● ***Waste Management Room***
Unit Chief: *MATSUDA, Yoshimi*

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

Research Support Group

Chief: *MIZUTANI, Takeshi*

● ***Cell Biology***
Unit Chief: *HAYASHI, Kohji*
Technical Staff: *BINO, Takahiro*
NISHIMOTO, Yuki

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

● ***Neurobiology***
Vice-Unit Chief: *TAKEUCHI, Yasushi*

● ***Evolutionary Biology and Biodiversity***
Unit Chief: *FUKADA-TANAKA, Sachiko*
Technical Staff: *OHI, Shoko*

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

Reception

Admin Support Staff: *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.

Collaboration Programs with Overseas Institutions

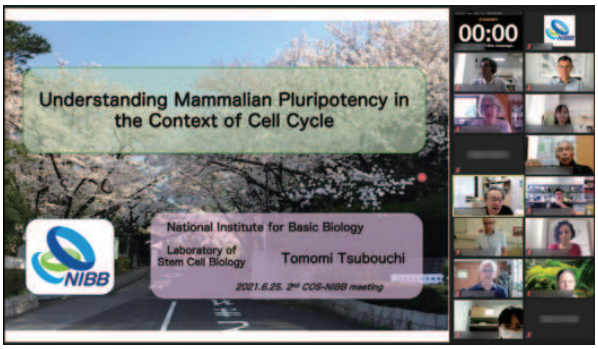
NIBB-Center for Organismal Studies (COS), Heidelberg, Germany

NIBB-COS international collaboration research project focusing on the mechanisms and evolution of light sensing in cnidarians

NIBB and COS Heidelberg started an international collaborative research project focusing on the mechanism and evolution of light sensing in cnidarians. This project was financially supported by the NINS. In this collaborative research project, a new emerging model organism, sea anemone (*Aiptasia* sp.), was used. Professor Annika Guse, who has been conducting research analyzing the mechanisms and evolution of light sensing in sea anemones at COS Heidelberg, joined the "Open Laboratory" established in NIBB. In October 2020, a post-doc researcher, Dr. Mariko Kishimoto who received PhD degree in NIBB, joined this research project and has been conducting research using molecular biological and molecular genetic approaches. More detail are provided on the page of the collaborative research project (p. 75).

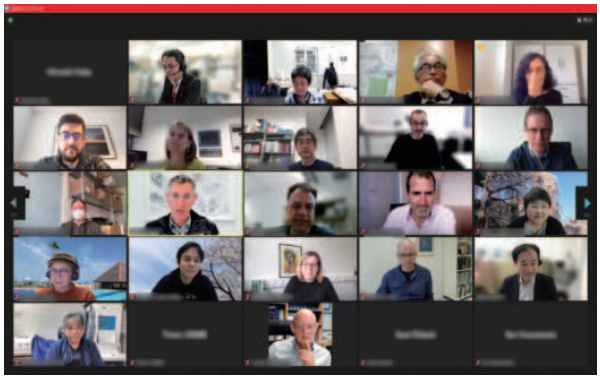
The meeting for NIBB-COS Heidelberg International Collaborations
Lecture series #2 "Stem Cell"
Lecture series #3 "Cell Signaling / Cell Biology"

The meeting for NIBB-COS Heidelberg International Collaborations was organized in conjunction with COS Heidelberg in Germany. During the Lecture Series held in this program, two researchers from both institutes gave a presentation on the specific theme. In the 2nd meeting held on June 25, 2021, the lecture series theme was "Stem Cell". Dr. Tomomi Tsubouchi from the Laboratory of Stem Cell Biology and Dr. Sergio P. Acebrón from COS Heidelberg talked about their research projects. Thirty-nine members from NIBB and 33 from COS Heidelberg (both totals included post-docs and students) participated in this event. The theme of the 3rd meeting held on March 31, 2022, was "Cell Signaling / Cell Biology". Dr. Kazuhiro Aoki from the Division of Quantitative Biology and Dr. Gislene Pereira from COS Heidelberg gave their presentations to 36 NIBB members and 26 COS members. During both events, lively discussions took place.



Lecture by Tomomi Tsubouchi (NIBB) in the 2nd meeting.

We also organized the social meetup events in the meetings and used the video chat tool, Spatial Chat, as an online meeting platform to facilitate it. Principal investigators, researchers, and students from both institutes were thus able to meet and have lively exchanges. By manipulating the specially assigned icon given to each of them, participants were able to freely join any discussions they chose to partake in with no limitations on the number of people involved. Consequently, the event was highly praised.



Group discussion after the lectures in the 3rd meeting.

In these meetings, several discussions on new collaborative research were put forward, and as a result, ties between NIBB and COS seemed to have deepened. Some participants suggested that the exchange of young researchers and students from both institutes would be considered and that a poster session would be held at the meeting. In light of these comments, we are considering holding an online poster session for the next meeting.

NIBB-Princeton University, USA

Collaborative activities between NIBB and Princeton University are conducted under the support of the International Research Collaboration Center (IRCC) of the National Institutes of Natural Sciences (NINS), based on the academic exchange agreement between the NINS and Princeton University. In FY2020, Dr. Ellen Reed, an IRCC's specially appointed research staff and postdoctoral research fellow of Princeton University, promoted a collaborative research project with Professor Kazuhiro Aoki of NIBB and Professor Jared Toettcher of Princeton University on "Dissolving biomolecular condensates using optical or chemical recruitment of soluble proteins" at Princeton University. In FY2021, due to the COVID-19 situation, it was difficult to travel between NIBB and Princeton University. However, collaborative research activities were conducted using online communication tools and we agreed that Dr. Reed would visit NIBB and carry out the collaboration in Prof. Aoki's laboratory in the next year.

Collaborative Activities with Universities and Research Institutes in Japan

NIBB-Institute of Low Temperature Science (ILTS), Hokkaido University

Accomplishment of the NIBB Priority Collaborative Research Project

■ Project Title:
Molecular and physiological mechanisms for understanding mammalian hibernation and their comparative analysis among species

■ Co-Principal Investigators:
Prof. Yoshifumi Yamaguchi (ILTS)
Prof. Toshihiko Fujimori (NIBB)
Prof. Shuji Shigenobu (NIBB)

NIBB-Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University

International Online Seminar by IMEG, Kumamoto University

IMEG at Kumamoto University has been holding a series of monthly seminars called "IMEG Seminar Series - The road to global science -" as part of its activities at the International Advanced Research Center. As part of the collaboration agreement between IMEG and NIBB, NIBB members has participated in this seminar series.

■ Seminar series:
2021 May: Jacqueline Tabler (Max Planck Institute of Molecular Cell Biology and Genetics, Germany)
2021 June: Vikas Trivedi (EMBL Barcelona)
2021 July: Patrick PL Tam (University of Sydney, Australia)
2021 Aug: Eran Meshorer (The Hebrew University of Jerusalem, Israel)
2021 Sep: Jacob H. Hanna (Weizmann Institute of Science, Israel)
2021 Oct: Kathy Niakan (The Francis Crick Institute and the University of Cambridge, UK)
2021 Nov: Xin Chen (Johns Hopkins University, Howard Hughes Medical Institute, USA)
2021 Dec: Peter K. Todd (University of Michigan, USA)
2022 Jan: Peter S. Zammit (King's College London, UK)
2022 Feb: John B. Wallingford (University of Texas at Austin, USA)
2022 Mar: Qi-Long Ying (University of South California, USA)

NIBB-Institute of Advanced Medical Sciences (IAMS), Tokushima University

NIBB- IAMS, Tokushima University, joint seminar "Toward Strengthening Collaboration and Cooperation"

Following the collaboration agreement between NIBB and IAMS, a joint seminar was held on October 22, 2021, to strengthen cooperation and budding new collaborative research between the two institutions. The seminar introduced the facilities and equipment of both institutes, as well as efforts for their joint usage/research programs. A total of 76 people attended the seminar, 49 from NIBB and 27 from IAMS.

After the seminar, an opinion exchange meeting was held with the participation of directors and faculty from both sides. They actively discussed future cooperation and collaboration in joint usage/research programs, the exchange of technologies and methods in which both sides have strengths, and training and exchanging of staff involved in the implementation and operation of the joint usage/research programs. This would be the first step toward deepening cooperation between NIBB and IAMS and leading to the development of collaborative research and joint usage/research programs between the two institutes in the future.

■ Seminar information:
Presentation from NIBB
Kiyokazu Agata (Introduction of NIBB)
Shuji Shigenobu (Introduction of NGS and mass spectrometry analyses)
Yasuhiro Kamei (Introduction of bioimaging analysis)
Eiji Watanabe (Introduction of model animal research facility)

■ Presentation from IAMS
Hidetaka Kosako (Introduction of mass spectrometry analysis)
Tomohide Saio (Introduction of nuclear magnetic resonance (NMR) analysis)
Yosuke Matsushita (Introduction of NGS analysis)
Tatsuya Takemoto (Introduction of genome editing analysis)

NIBB-Institute for Molecular and Cellular Regulation, Gunma University

Agreement of Cooperation with IMCR, Gunma University

NIBB and IMCR at Gunma University have signed an agreement of cooperation which is deemed effective from April 7, 2021. To prevent the further spread of COVID-19 infection, the signing ceremony was held in a remote capacity via an online connection between Maebashi and Okazaki.

Dr. Kiyokazu Agata, the Director-General of NIBB, and Dr. Ken Sato, the Director of IMCR, singing the agreement.

This agreement aims to promote mutual collaborative activities in the capacity of research and education centers that lead the way in world class, cutting edge research in the field of endocrinology, metabolism research and basic biology, as well as to utilize the results of these activities for research on a mutual basis. It also strengthens the support infrastructure for collaborative use and research nationwide, and to stimulate international academic exchange.

As part of the collaboration agreement, Director-general Kiyokazu Agata and Prof. Takashi Ueda present their research activities in the 7th IMCR Symposium that was held at September 9-10.

NIBB-Chubu University

The 3rd Chubu University-NIPS-NIBB joint seminar "AI to Life System"

NIBB and NIPS have been promoting exchanges of personnel and information with Chubu University through joint seminars on the theme of "AI to Life System" since the fall of 2021, aiming for emergence through interdisciplinary research and the discovery and cultivating of young researchers. The first joint seminar was hosted by Chubu University in November 2021, and the 2nd seminar was organized by NIPS in January 2022.

On March 28, 2022, NIBB organized the 3rd joint seminar held as a hybrid meeting with Zoom online system at the Okazaki Conference Center. In addition to the lectures of ongoing research utilizing AI analysis methods at each institute, a small group discussion was held to promote new collaborative research between the three institutes. The group discussions were divided into two categories, "technology" and "science". In each category, four topics were set up, and researchers and students discussed each topic separately. This event helped to deepen mutual understanding between information science and life science, and promoted the budding of collaboration research leading to the "elucidation of life systems using AI.

Opening remarks by Dr, Kiyokazu Agata, the Director-General of NIBB.

Group discussion on the topics of gene and protein.

- Seminar information
  Lectures
  Takashi Ueda (NIBB)
  Junichi Chikazoe (NIPS)
  Seine Shintani (Chubu University)
- Group discussion
  1) Technology: Computer vision, Robotics, fMRI, and Electron Microscopy.
  2) Science: Gene and protein, Cell, Brain and sensory, and Brain and behavior.

The NIBB Genome Informatics Training Course

The NIBB Core Research Facilities regularly organizes a series of training courses on the most recently developed research techniques. The NIBB Genome Informatics Training Course (GITC) is specially designed for biologists who are unfamiliar with bioinformatics. In 2021, we held two sets of training courses on RNA-seq analysis. Each version of the RNA-seq analysis course was basically made up of two 2-day programs: one being a preparatory course (Introduction to NGS Analysis) concerning the basics of UNIX and R, and the other a practical course (Introduction to RNA-seq) for learning about the pipelines to RNA-seq analysis using next-generation sequencing data. These GITC courses offered lectures and hands-on tutorials. This year, all courses were held online to prevent the spread of COVID-19 infection. By virtue of the online system, we expanded the course audiences by accepting some of them as "auditors" who could receive only limited support during the hands-on practice.

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. Hiroki Sugiura (NIBB Core Research Facilities)

August 25 (Thu)–26 (Fri), 2021

(Practical Course) Introduction to NGS Analysis: Basics of UNIX, R, and NGS

- 31 participants and 16 auditors (including 2 from NIBB)
- Program:
  1. UNIX for Beginners
  2. Introduction to "R"
  3. Introduction to Statistics
  4. NGS Basic Data Formats and NGS Basic Tools
  5. Editor and Scripts
  6. Text Processing
  7. Exercises

September 15 (Thu)–16 (Fri), 2021

(Practical Course) RNA-seq Analysis Pipeline

- 28 participants and 16 auditors (including 3 from NIBB)
- Program:
  1. Introduction to RNA-seq
  2. NGS Basic Data Format and Basic Tools
  3. Visualization of NGS Data
  4. RNA-seq Pipelines: Genome-Based and Transcriptome-Based Approaches
  5. Multivariate Statistics
  6. Functional Annotation and Gene Ontology
  7. Exercises

Introduction to NGS Analysis: Basics of UNIX, R, and NGS

February 9 (Wed)–10 (Thu), 2022

(This program was the same as the above listed practical course)

■ 27 participants and 16 auditors (including 5 from NIBB)

Introduction to RNA-seq: RNA-seq Analysis Pipeline

March 2 (Wed)–3 (Thu), 2022

(This program was the same as the above listed practical course)

■ 27 participants and 12 auditors (including 5 from NIBB)

The Bio-imaging Data Analysis Training Course 2021

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

November 24 (Wed)–26 (Fri), 2021

The 9th Bio-imaging Data Analysis Training Course was jointly held 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 was related to learning about image processing and analytical techniques through solving simple problems with image analysis, and understanding appropriate methods and necessary preparation when consulting experts in technically advanced problems concerning imaging. 134 people applied for the course, which had an announced capacity of 16 participants. Given the high demand for courses on these subjects, we accepted 33 participants.

This course's lectures were conducted with the aim of guiding participants towards an awareness of the series of steps essential for fundamental image processing and analysis while also obtaining images for eventual use (workflows). In addition to this, participants independently worked on practical image analysis exercises using ImageJ; a typical open-source software package for biological image processing and analysis. Lectures were also given on how the programming of simple “macro language”, which uses the aforementioned workflows in ImageJ, allows for automation; a necessity for the large capacity and high-dimensional throughput of microscopic imaging which has more become common over recent years.

Upon the course’s conclusion, each student offered commentary about and discussed the methods taught by providing examples of images gleaned from their own research.

Unfortunately, although this year's course was held online due to issues associated with COVID-19, we utilized technological innovations within the lectures and exercises, such as a chat service to support individual participants, so as to allow them to feel as if the course was being held as an in-person event.

Every year following the course’s completion, participants often report feeling pretty tired, but satisfied. In a similar vein, we believe that this course is beneficial to its participants thanks to the associated increase in their familiarity with image analysis techniques. Thus, we expect that this course will increase opportunities for joint research relating to biological image analysis moving forward.

(Kagayaki Kato)

Advanced Bioimaging Support (ABiS)

The demand for bioimaging has increased in recent years in the field of life science. However, due to advances in imaging technologies, such as the diversification and specialization of imaging equipment, 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 Prof. Naoto UENO and Assist. Prof. Hiroshi KOYAMA.

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.

Since the ABiS project that spanned 6 years was finished at the end of this fiscal year, we self-evaluated the past ABiS activities and forecast the future demands, and applied for the Grant-in-Aid for Transformative Research Areas, Platforms for Advanced Technologies and Research Resources (FY2022) as the next ABiS project. In February 2022, our past activities were highly evaluated by the committee and plans for the next project have been approved for the second phase of the ABiS project (FY2022-FY2027). As in the past, the ABiS will continue to support light microscopy, electron microscopy, magnetic resonance imaging, and image analysis as the core institute together with the Institute for Physiological Sciences.

ABiS Symposium "Image Data Analysis to Open Up A New Era for Life Science"

March 1 (Tue) 2022

The ABiS symposium "Imaging Data Analysis to Open Up A New Era for Life Science" was held this year as an online event. The following 8 researchers, experts in image data analysis and database construction, gave lectures.

- Naomi KAMASAWA (The Max Planck Florida Institute for Neuroscience Imaging Center)
- Nobuhiko OHNO (Jichi Medical University/ National Institute for Physiological Sciences)
- Tatsumi HIRATA (National Institute of Genetics)
- Kazuo IABA (Tsukuba University)
- Naohiro OKADA (The University of Tokyo)
- Saori TANAKA (Brain Information Communication Research Laboratory Group)
- Shu-ichi ONAMI (RIKEN Center for Biosystems Dynamics Research)
- Yoshitaka KIMORI (Fukui University of Technology)

There were 168 participants, and a lively question and answer session was held for each presentation. A participant survey and other information about this symposium is available on the website. (https://www.nibb.ac.jp/abis/ev20220301/)

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 FY2021, considering the situation of COVID-19, we called the applications from students attending universities abroad with the explicit understanding that the internship program might cancel if the COVID-19 situation worsened. Consequently, one student who belongs to a Vietnamese university was selected. However, due to border control by the Japanese Government, the student was unable to visit Japan. In contrast, four international students attending universities in Japan were accepted, and three of them came to NIBB during FY2021.

Report from a participant
Piyusha Mongia

I am Piyusha Mongia, a graduate student from Osaka University, Japan. I am grateful that I could take part in this internship program and be a part of Prof. Jun-Ichi Nakayama's laboratory. The lab works on epigenetics- how it regulates gene expression, the establishment of epigenetic markers and so on.

The bigger question was to understand how epigenetic information is inherited and what are the factors involved. Epigenetics refers to heritable changes- not coded by DNA. Eukaryotic chromosomes can be divided into 2 broad categories: euchromatin and heterochromatin. Euchromatin refers to the regions on chromosomes which are transcriptionally active. Heterochromatin, on the hand, is generally transcriptionally silent, present at repetitive sequences or transposons. Methylation of lysine 9 of histone H3 (H3K9me) is a hallmark of heterochromatin. I created and assessed the involvement of four candidate gene deletions in heterochromatin inheritance in fission yeast spores. Fission yeast is a simple eukaryote having three chromosomes, making its study relatively easier. I first replaced the 4 genes with nourseothricin by PCR and then visualized swi6-egfp localization by fluorescence microscopy. One of the candidate genes seems to be important for the inheritance of heterochromatin but more studies need to be done to conclude so. In a parallel project, I tried to express three of Tetrahymena proteins using a bacterial expression system. These proteins are involved in heterochromatin assembly. Producing recombinant proteins allows us to purify and biochemically analyze individual proteins activities and interactions in-vitro. In the end, I managed to express all, but purify one of the proteins due to time constraints. I learned many biochemical techniques, like Gel Filtration Chromatography and Ion Exchange Chromatography. I wish I could have had a longer time period to do more fun experiments.

Every day I was faced with challenges, and learnt something new. All the members were very patient with me and taught me something or the other. I'm extremely grateful to Dr. Hayashi and Dr. Kataoka, who guided me throughout the project. Prof. Nakayama was very supportive and encouraging. The research atmosphere in the lab is very exciting and it was truly an enriching experience for me.

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 Elementary and Junior High Schools

NIBB, in collaboration with the Okazaki City Board of Education, visited elementary and junior high schools during 2021 to give special classes.

October 13 (Wed), 2021

Special class at Ryukai Junior High School

■ Prof. Toshihiko Fujimori

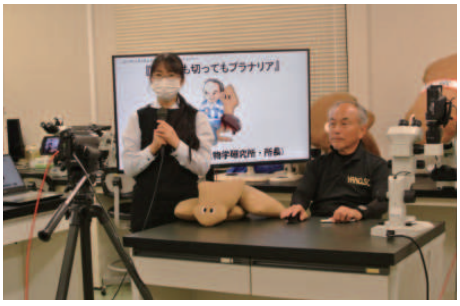
"The Beginning of Forming Animals - Thinking from the Cell"

November 9 (Tue), 2021

Special class to All junior high schools in Okazaki (online)

■ Director General Kiyokazu Agata

"Planarian No Matter How Many Cuts"



November 10 (Wed), 2021

Special class at Iwazu Junior High School

■ Prof. Teruyuki Niimi

"Full of Wonders in Diverse Insects"

November 15 (Mon), 2021

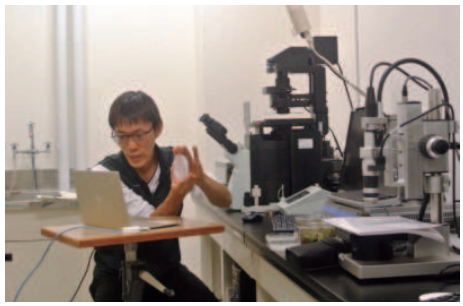
Special class to Tokiwa Junior High School (online)

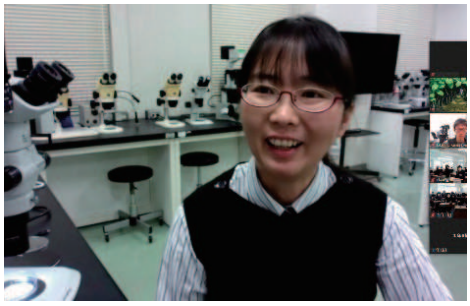
■ S. A. Assist. Prof. Tomoko Kurata

"Virtual Tour in NIBB"

■ Assist. Prof. Takehiko Kanazawa

"The Micro World of Plants"



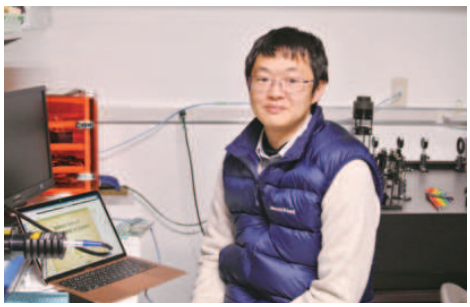


February 14 (Mon), 2022

Special class to Atago elementary school (online)

■ Assoc. Prof. Makio Yokono

"How Do Plants Use Light to Build Bodies?"



Work experience for junior high school students at NIBB

Kita Junior High School

Visit to High Schools

February 17 (Thu), 2022

Special class at Okazaki High School

■ Prof. Miyo Morita T.

"Plants that Rise Up After Falling Down: Mechanism of Gravity Sensing"

July 2 (Fri), 2021

Special class at Okazaki Kita High School

■ Assoc. Prof. Nobuyuki Shiina

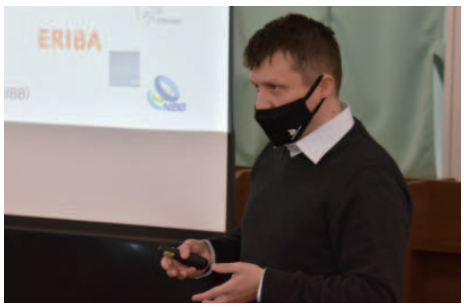
"Mechanism of Long-Term Memory Formation"

March 4(Fri), 2022

Special class at Okazaki Kita High School

■ Dr. Yakub Wudarski

"Science and English"



June 22 (Tue), 2021

Special class at Meiwa High School

■ Prof. Teruyuki Niimi

"Exploring the Diversity of Insect Patterns and Forms"

July 27 (Tue), 2021

Special class to Tondabayashi High School (online)

■ S. A. Assist. Prof. Tomoko Kurata

October 27 (Wed), 2021

Special class to Kanagawa Hakuyo High School (online)

■ S. A. Prof. Kiyoshi Naruse

"Where Did Medaka Came From?"

November 2 (Tue), 2021

Special class at Aichi Sangyo University Mikawa High School

- Prof. Teruyuki Niimi

"Exploring the Diversity of Insect Patterns and Forms"

August 20(Fri),2021
December 24(Fri),2021

Feedback session regarding research presentations by high school students (online)

- S. A. Assist. Prof. Kiyoshi Tatematsu

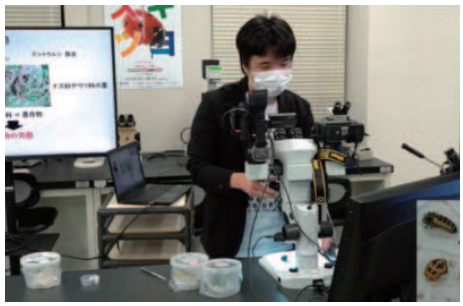
Programs broadcast live online

April 24 (Sat)– May 1 (Sat), 2021
May 21 (Fri), 2021 (Sequel)

Live broadcast at niconico live

"Watching the complete metamorphosis of ladybirds."

- Director General Kiyokazu Agata
- Prof. Teruyuki Niimi
- Prof. Shuji Shigenobu
- Assis. Prof. Toshiya Ando
- Graduate Student Yasuhiko Chikami
- S. A. Assist. Prof. Tomoko Kurata



January 15 (Sat)– January 16 (Sun), 2022
January 28 (Fri), 2022 (Sequel)

Live broadcast at niconico live

- Director General Kiyokazu Agata
- Graduate Student Miyuki Ishida
- S. A. Assist. Prof. Tomoko Kurata

"Is it possible to hijack and regenerate? —Transplanting planarian stem cells."

Lectures for the public

September 25(Sat), 2021

Public lecture at the Kihara Institute of Biology

- Assist. Prof. Atsushi Hoshino

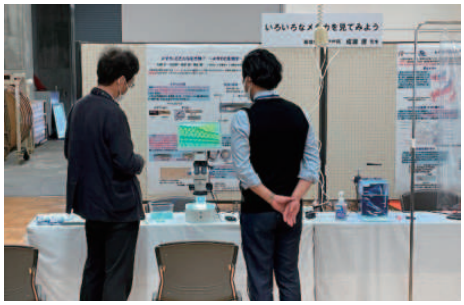
"How to Create the One and Only Morning Glory in the World"

March 27 (Sun), 2022

Public lecture at the event held by Ohsumi Frontier Science Foundation

- S. A. Prof. Kiyoshi Naruse

"Biodiversity and Utilization of Wild Medaka and Related Species —Perspectives from Biogenetic Resources"



July 20 (Tue), 2021

National Institute of Natural Sciences The 9th Young Researcher Award Commemorative Lecture (online)

- Assist. Prof. Kim Eunchul

"Even Plants Feel Dazzling at Times"

August 21 (Sat), 2021

The 32nd National Institutes of Natural Sciences Symposium (online)

■ S. A. Assist. Prof. Yoko Otsubo

"Close Encounters of Yeast with Plasma"

October 24 (Sun), 2021

Inter-university Research Institute Symposium 2021 (online)

■ Prof. Shin-ichi Higashijima

"Exploring the Mechanisms for Sensing Balance and Postural Control"

February 24 (Wed)–March 31 (Thu), 2022

Presentation by OKASHIN (Okazaki Credit Union) scholarship students (online)

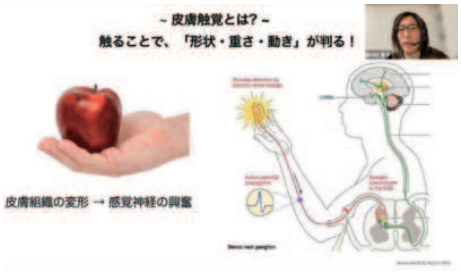
■ Graduate Student Masamichi Ueda

February 25 (Fri), 2022

Online lecture by NIBB and NIPS

"The discovery of the PIEZO channel that solved the mystery of skin sensation and the world of mechanosensing research opened up through them"

■ Assoc. Prof. Keiko Nonomura



Exhibition support

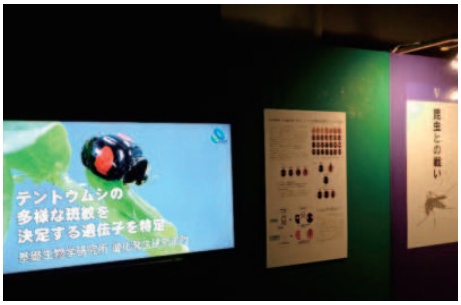
July 9 (Fri)–September 5 (Sun), 2021

Toyohashi Museum of Natural History

The 35th Special exhibition "The Wonderful World of Insects"

■ Prof. Teruyuki Niimi

■ S. A. Assist. Prof. Tomoko Kurata



July 10 (Sat)–September 20 (Mon), 2021

National Museum of Nature and Science, Tokyo

January 14 (Fri)–April 3 (Sun), 2022

Osaka Museum of Natural History

Special exhibition "PLANTS Mainstays of the Planet"

■ Prof. Mitsuyasu Hasebe (Supervisory support)

■ Prof. Takashi Ueda

■ Assist. Prof. Atsushi Hoshino

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

Take the South Exit of the station and walk approximately 7 minutes to Myodaiji-area, 20 minutes to Yamate-area.

By Car

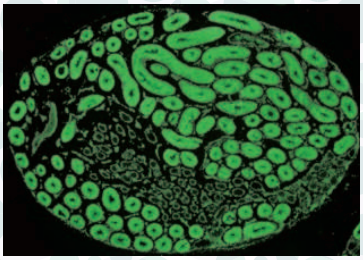
Take the Tomei Expressway to the Okazaki Exit.
Turn left at the City Hall S. signal
(approximately 10 minutes from the exit).

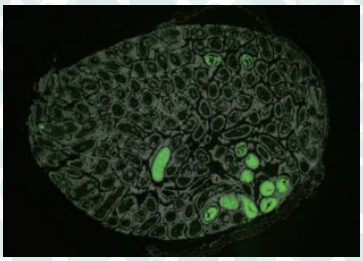
Myodaiji-area

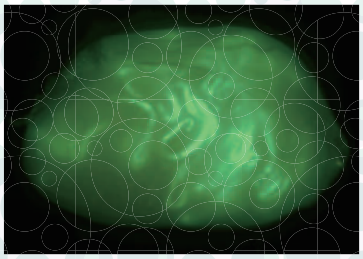
38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585 Japan

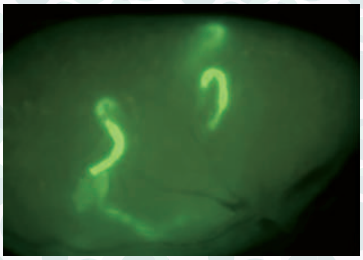
Yamate-area

5-1 Higashiyama, Myodaiji, Okazaki, Aichi 444-8787 Japan









National Institutes of Natural Sciences
National Institute for Basic Biology

38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585 Japan
phone: +81 564-55-7000 fax: +81 564-53-7400
https://www.nibb.ac.jp/en/
Issued in December 2022
Edited by the Research Enhancement Strategy Office, NIBB