

National Institute for Basic Biology

# 2020 ANNUAL REPORT

April 2020–March 2021

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The cover items are related to a paper titled “Calcium dynamics during trap closure visualized in transgenic Venus flytrap” (Suda *et al.*, Nature Plants 2020) from Division of Evolutionary Biology. See page 47 of this report for details.

It is my great pleasure to present to you all the National Institute for Basic Biology (NIBB)'s 2020 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 2020 to March 2021.

NIBB's activities in 2020 were severely affected by the COVID-19 pandemic. All planned international conferences were postponed and all international collaborative activities were switched to an online-based format. The first joint meeting between NIBB and The Centre for Organismal Studies (COS) Heidelberg was also held online in March 2021. Although researcher interactions within the country were also limited, we could maintain research activities within the institute at almost normal levels.

Concerning changes on the staffing front, Associate Professor Ryuji Kodama retired in March 2021 and Assistant Professor Ikuo Uchiyama was promoted to Associate Professor in April 2020. Additionally, one specially appointed associate professor, four assistant professors and fourteen specially appointed assistant professors were newly posted to NIBB during this year.

I would like to finish this introduction by congratulating Professor Masayoshi Kawaguchi, who was awarded the 2020 Japanese Society of Plant Physiologists Award, and Professor Teruyuki Niimi, who was awarded the 2020 Japanese Society of Sericulture Science Award.

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

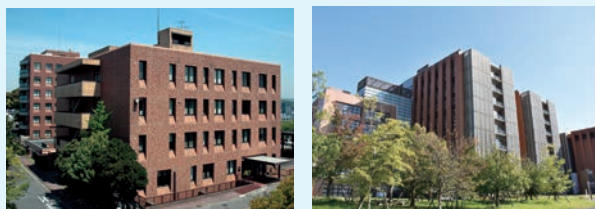
Kiyokazu Agata  
Director General of NIBB  
December, 2021





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 and four other national institutes, the National Astronomical Observatory of Japan (NAOJ), the National Institute for Fusion Science (NIFS), the National Institute for Physiological Sciences (NIPS), and the Institute for Molecular Science (IMS), constitute the National Institutes of Natural Sciences (NINS), which is one of the four Inter-University Research Institute Corporations. NIBB's activities are supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.



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

## Policy, Decision Making and Administration

The Director General oversees the operation of NIBB 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 in order to improve NIBB's abilities as a collaborative research institution.

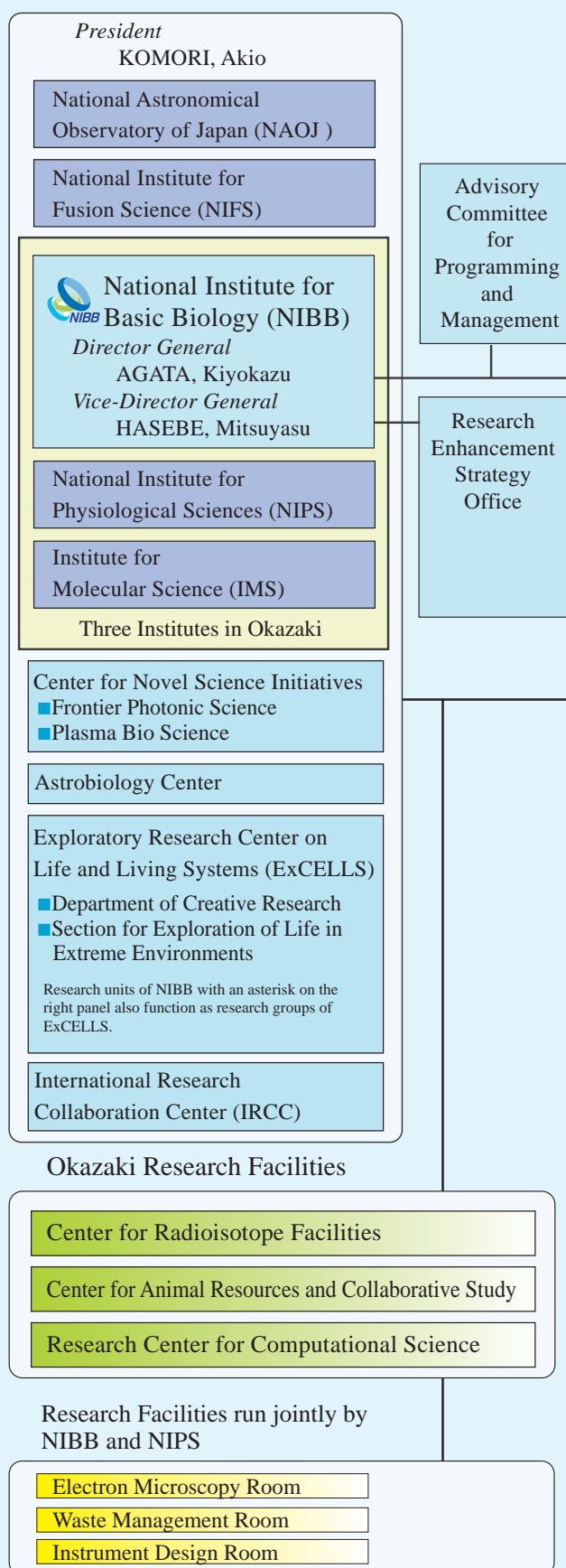
Administration of NIBB is undertaken by the Okazaki Administration Center under the direct auspices of the Administration Office of NINS.

## Research and Research Support

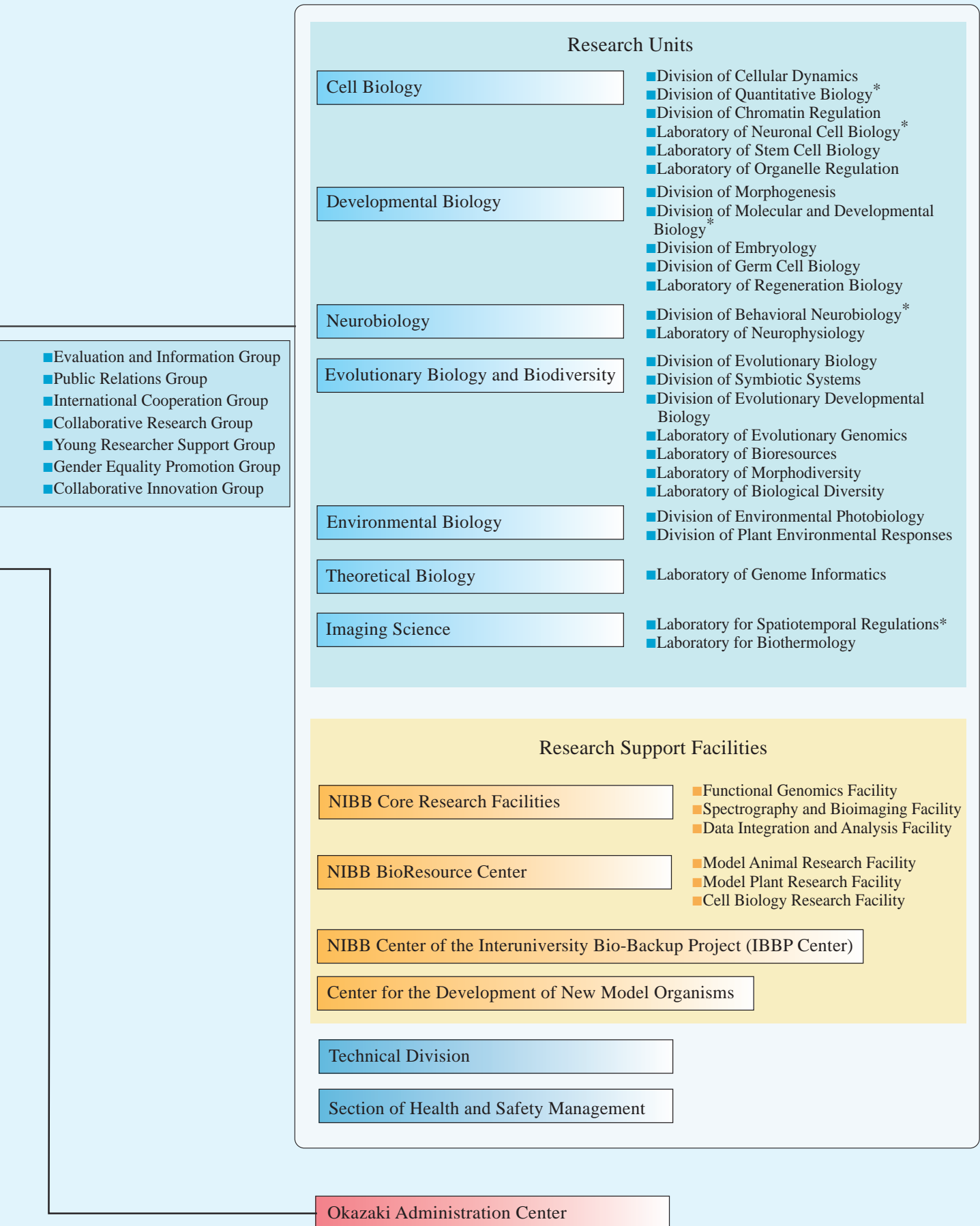
NIBB's research programs are conducted in research units called divisions and laboratories. Each research unit forms an independent project team. The NIBB Core Research Facilities and NIBB BioResource Center were launched in 2010 to support research at NIBB and also to act as an intellectual hub to promote 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

## Organization

### National Institutes of Natural Sciences (NINS)



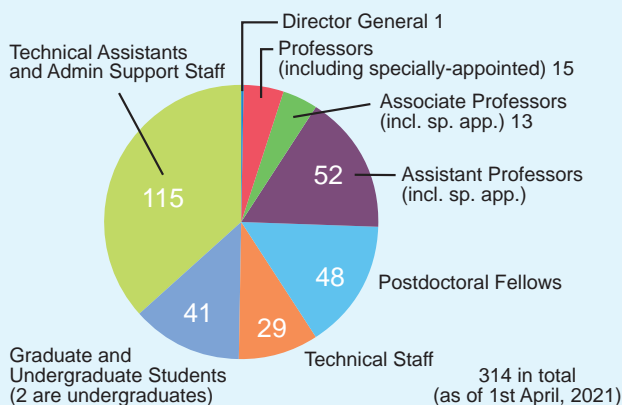




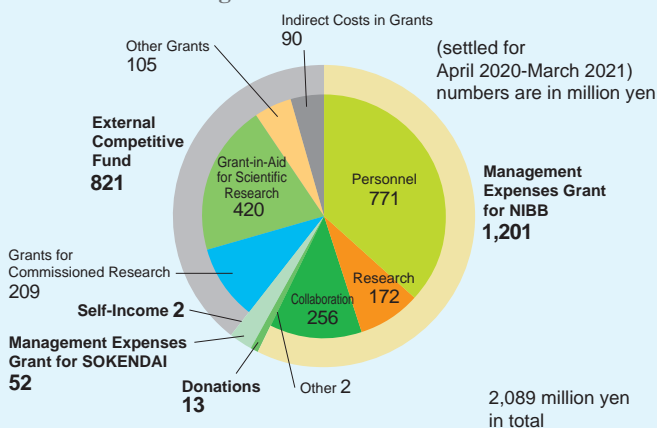
staff and helps to promote the activities of each research unit and research support facility to maintain the common resources of NIBB and NINS.

The Astrobiology Center (ABC) was established in 2015 as an 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



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

| Committee                  | Name                   | Position  |
|----------------------------|------------------------|---|
| External committee members | HANASHIMA, Carina      | Professor, Waseda University                                |
|                            | HIRAOKA, Yasushi ##    | Professor, Osaka University                                 |
|                            | KITANO, Jun            | Professor, National Institute of Genetics                   |
|                            | KOHCHI, Takayuki       | Professor, Kyoto University                                 |
|                            | KUROIWA, Asato         | Professor, Hokkaido University                              |
|                            | SATAKE, Akiko          | Professor, Kyushu University                                |
|                            | SIOMI, Mikiko          | Professor, The University of Tokyo                          |
|                            | SUGIMOTO, Asako        | Professor, Tohoku University                                |
|                            | YAMAMOTO, Takashi      | Professor, Hiroshima University                             |
|                            | YOSHIMURA, Takashi     | Professor, Nagoya University                                |
| Internal committee members | AOKI, Kazuhiro         | Professor, ExCELLS and National Institute for Basic Biology |
|                            | FUJIMORI, Toshihiko    | Professor, National Institute for Basic Biology             |
|                            | HASEBE, Mitsuyasu      | Professor, National Institute for Basic Biology             |
|                            | HIGASHIJIMA, Shin-ichi | Professor, ExCELLS and National Institute for Basic Biology |
|                            | KAWAGUCHI, Masayoshi # | Professor, National Institute for Basic Biology             |
|                            | MINAGAWA, Jun          | Professor, National Institute for Basic Biology             |
|                            | NIIMI, Teruyuki        | Professor, National Institute for Basic Biology             |
|                            | TAKADA, Shinji         | Professor, ExCELLS and National Institute for Basic Biology |
|                            | UEDA, Takashi          | Professor, National Institute for Basic Biology             |
|                            | UENO, Naoto            | Professor, National Institute for Basic Biology             |
|                            | YOSHIDA, Shosei        | Professor, National Institute for Basic Biology             |

# Chairperson  
## Vice-Chair

## GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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. "Priority Collaborative Research Projects" are carried out as group research projects by internal and external researchers to develop pioneering research fields. 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  | 2018       | 2019       | 2020       |
|---|------------|------------|------------|
| Priority collaborative research projects  | 1          | 1          | 1          |
| Collaborative research projects for new model organism development                  | —          | —          | 2          |
| Collaborative research projects for model organism and technology development       | 2          | 2          | —          |
| Individual collaborative research projects  | 57         | 60         | 46         |
| Collaborative research projects for integrative genomics                            | 67         | 66         | 56         |
| Collaborative research projects for integrative bioimaging                          | 23         | 22         | 19         |
| NIBB workshops  | 2          | 3          | 2          |
| Collaborative experiments using the Large Spectrograph                              | 9          | 9          | 9          |
| Priority collaborative research projects  | 1          | 0          | 0          |
| Collaborative research projects for bioresource preservation technology development | 18         | 14         | 10         |
| <b>Total</b>  | <b>180</b> | <b>177</b> | <b>145</b> |

#### ■ 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 (*Oryzias 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. 99-100).

#### ■ Interuniversity Bio-Backup Project for Basic Biology (IBBP)

To prevent damage caused by natural disasters to important biological resources, NIBB has managed the Interuniversity Bio-Backup Project for Basic Biology (IBBP) from 2012 in collaboration with seven national universities for multiple preservation of genetic libraries and other invaluable bioresources used in cutting-edge research (p. 101-102).

#### ■ 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. 114-115).

### International Cooperation

#### ■ Collaborative Programs with Overseas Institutes

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

NIBB has formed an agreement with Princeton University and COS Heidelberg to promote joint research projects, collaborative symposia, training courses and student exchange programs. The Kick Off (1st) Meeting for NIBB COS Heidelberg International Collaborations was held in March 2021 (p. 110).

#### ■ 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.

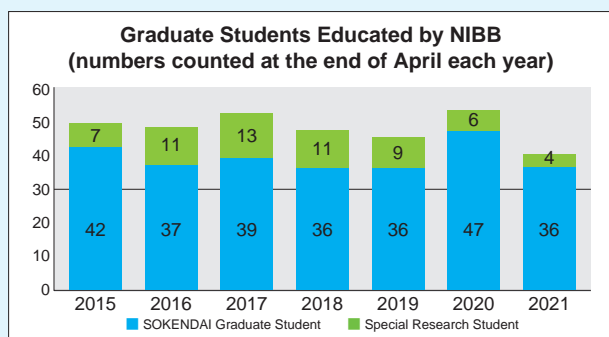
## Cultivation of Future Researchers

### ■ 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. 116).



### ■ 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). Streaming live videos concerning the development of model organisms have been viewed numerous times. NIBB also cooperates in the education of undergraduate and young students through lectures and workshops (p. 117-119).

## ■ Personnel changes from April 2020 to March 2021\*

### Newly assigned to NIBB

| Name                | Position                                | Research Unit  | Date            |
|---------------------|---|--|-----------------|
| UCHIYAMA, Ikuo      | Associate Professor                     | Laboratory of Genome Informatics<br>Data Integration and Analysis Facility | April 1, 2020   |
| SEGAMI, Shoji       | Assistant Professor                     | Division of Evolutionary Biology   | April 1, 2020   |
| HIRA, Seiji         | Specially Appointed Assistant Professor | Division of Germ Cell Biology  | April 1, 2020   |
| MORITA, Shinichi    | Specially Appointed Assistant Professor | Division of Evolutionary Developmental Biology                             | April 1, 2020   |
| SAKAMOTO, Joe       | Specially Appointed Assistant Professor | Laboratory for Biothermology   | April 1, 2020   |
| KOSETSU, Ken        | Specially Appointed Assistant Professor | Division of Evolutionary Biology   | April 1, 2020   |
| MINAMINO, Naoki     | Specially Appointed Assistant Professor | Division of Cellular Dynamics  | April 1, 2020   |
| YOKE, Hiroshi       | Specially Appointed Assistant Professor | Laboratory for Spatiotemporal Regulations                                  | April 1, 2020   |
| KOBAYASHI, Taisuke  | Specially Appointed Assistant Professor | Laboratory of Neurophysiology  | April 1, 2020   |
| SHINOZUKA, Takuma   | Specially Appointed Assistant Professor | Division of Molecular and Developmental Biology                            | April 1, 2020   |
| LIU, Meng           | Specially Appointed Assistant Professor | Division of Symbiotic Systems  | April 1, 2020   |
| HAYASHI, Aki        | Specially Appointed Assistant Professor | Division of Chromatin Regulation   | April 1, 2020   |
| KAWAMOTO, Nozomi    | Specially Appointed Assistant Professor | Division of Plant Environmental Responses                                  | April 1, 2020   |
| KURASHIMA, Kiminori | Specially Appointed Assistant Professor | Laboratory of Stem Cell Biology  | May 1, 2020     |
| HUANG, Jia-Hsin     | Assistant Professor                     | Laboratory of Evolutionary Genomics  | June 1, 2020    |
| SAITO, Nen          | Specially appointed Associate Professor | Laboratory of Biological Diversity   | August 1, 2020  |
| WUDARSKI, Jakub     | Specially Appointed Assistant Professor | Laboratory of Biological Diversity   | August 1, 2020  |
| YODA, Shinichi      | Specially Appointed Assistant Professor | Laboratory of Evolutionary Genomics  | October 1, 2020 |
| OHASHI, Rie         | Assistant Professor                     | Laboratory of Neuronal Cell Biology  | January 1, 2021 |
| KIM, Eunuchul       | Assistant Professor                     | Division of Environmental Photobiology                                     | January 1, 2021 |

### Newly affiliated with other universities and institutes

| Name                | New Affiliation                | Position                                | Date               |
|---------------------|--------------------------------|---|--------------------|
| NAKAYAMA, Kei       | Kobe Pharmaceutical University | Assistant Professor                     | September 30, 2020 |
| TAKAHASHI, Shunichi | University of the Ryukyus      | Professor                               | February 1, 2021   |
| MORITA, Shinichi    | Nagoya University              | Specially Appointed Assistant Professor | March 1, 2021      |
| HUANG, Jia-Hsin     | AI Labs (Taiwan)               | Scientist                               | April 1, 2021      |
| TOKUTSU, Ryutaro    | Kyoto University               | Program-Specific Researcher             | April 1, 2021      |

### Mandatory Retirement

| Name          | Position            | Research Unit                 | Date           |
|---------------|---------------------|-------------------------------|----------------|
| KODAMA, Ryuji | Associate Professor | Laboratory of Morphodiversity | March 31, 2021 |

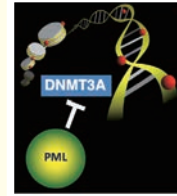
## ■ Awardees from January 2020 to March 2021

| Name                 | Position                                | Award   |
|----------------------|---|---|
| KAWAGUCHI, Masayoshi | Professor                               | Japanese Society of Plant Physiologists (JSPP) Award 2020 |
| NIIMI, Teruyuki      | Professor                               | Japanese Society of Sericulture Science Award 2020        |
| OTSUBO, Yoko         | Specially Appointed Assistant Professor | NINS Young Researcher Award                               |

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

**Genomic profiling by ALaP-Seq reveals transcriptional regulation by PML bodies through DNMT3A exclusion**

Kurihara, M., Kato, K., Sanbo, C., Shigenobu, S., Ohkawa, Y., Fuchigami, T., and Miyanari, Y. (2020). *Molecular Cell* 78.  
DOI:10.1016/j.molcel.2020.04.004



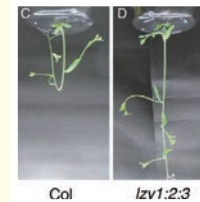
**Genomes of the venus flytrap and close relatives unveil the roots of plant carnivory**

Palfalvi, G., Hackl, T., Terhoeven, N., Shibata, T.F., Nishiyama, T., Ankenbrand, M., Becker, D., Förster, F., Freund, M., Iosip, A., Kreuzer, I., Saul, F., Kamida, C., Fukushima, K., Shigenobu, S., Tamada, Y., Adamec, L., Hoshi, Y., Ueda, K., Winkelmann, T., Fuchs, J., Schubert, I., Schwacke, R., Al-Rasheid, K., Schultz, J., Hasebe, M., Hedrich, R. (2020). *Current Biology* 30.  
DOI:10.1016/j.cub.2020.04.051



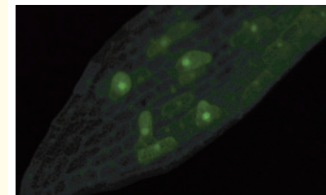
**Gravity-sensing tissues for gravitropism are required for “anti-gravitropic” phenotypes of *Lzy* multiple mutants in *Arabidopsis***

Kawamoto, N., Kanbe, Y., Nakamura, M., Mori, A., and Morita, M.T. (2020). *Plants* 9.  
DOI:10.3390/plants9050615



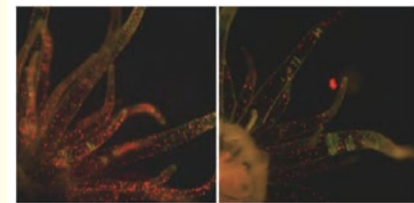
**DNA damage triggers reprogramming of differentiated cells into stem cells in *Physcomitrella***

Gu, N., Tamada, Y., Imai, A., Palfalvi, G., Kabeya, Y., Shigenobu, S., Ishikawa, M., Angelis, K.J., Chen, C., and Hasebe, M. (2020). *Nature Plants* 6.  
DOI:10.1038/s41477-020-0745-9



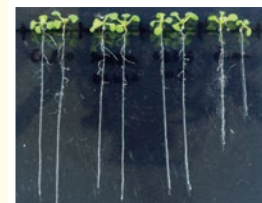
**Loss of symbiont infectivity following thermal stress can be a factor limiting recovery from bleaching in cnidarians**

Kishimoto, M., Baird, A.H., Maruyama, S., Minagawa, J., and Takahashi, S. (2020). *ISME Journal* 14.  
DOI:10.1038/s41396-020-00742-8



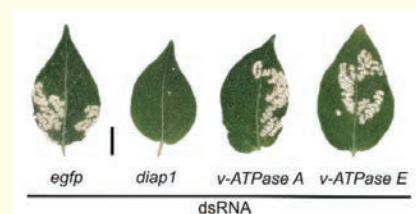
**Longin R-SNARE is retrieved from the plasma membrane by ANTH domain-containing proteins in *Arabidopsis***

Fujimoto, M., Ebine, K., Nishimura, K., Tsutsumi, N., and Ueda, T. (2020). *Proceedings of the National Academy of Sciences of the United States of America* 117.  
DOI:10.1073/pnas.2011152117



**Oral RNAi of *diap1* results in rapid reduction of damage to potatoes in *Henosepilachna vigintioctopunctata***

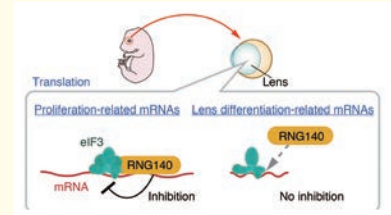
Chikami, Y., Kawaguchi, H., Suzuki, T., Yoshioka, H., Sato, Y., Yaginuma, T., and Niimi, T. (2021). *Journal of Pest Science* 94.  
DOI:10.1007/s10340-020-01276-w





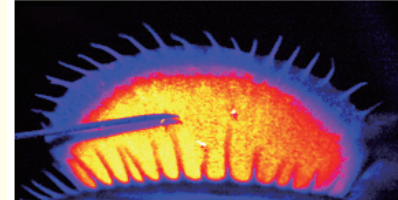
## Implications of RNG140 (caprin2)-mediated translational regulation in eye lens differentiation

Nakazawa, K., Shichino, Y., Iwasaki, S., and Shiina, N. (2020). *Journal of Biological Chemistry* 295. DOI:10.1074/jbc.RA120.012715



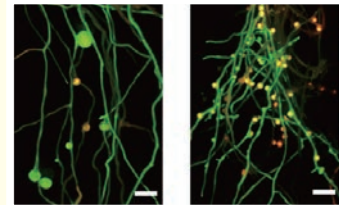
## Calcium dynamics during trap closure visualized in transgenic Venus flytrap

Suda, H., Mano, H., Toyota, M., Fukushima, K., Mimura, T., Tsutsui, I., Hedrich, R., Tamada, Y., and Hasebe, M. (2020). *Nature Plants* 6. DOI:10.1038/s41477-020-00773-1



## MIR2111-5 locus and shoot-accumulated mature miR2111 systemically enhance nodulation depending on HAR1 in Lotus japonicus

Okuma, N., Soyano, T., Suzuki, T., and Kawaguchi, M. (2020). *Nature Communications* 11. DOI:10.1038/s41467-020-19037-9



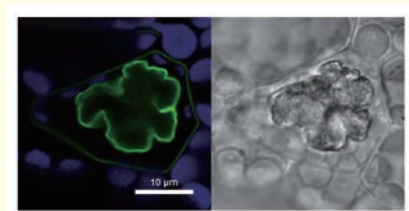
## A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, Acropora tenuis

Sakai, Y., Kato, K., Koyama, H., Kuba, A., Takahashi, H., Fujimori, T., Hatta, M., Negri, A.P., Baird, A.H., and Ueno, N. (2020). *Scientific Reports* 10. DOI:10.1038/s41598-020-74649-x



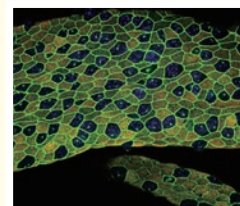
## The liverwort oil body is formed by redirection of the secretory pathway

Kanazawa, T., Morinaka, H., Ebine, K., Shimada, T.L., Ishida, S., Minamino, N., Yamaguchi, K., Shigenobu, S., Kohchi, T., Nakano, A., et al. (2020). *Nature Communications* 11. DOI:10.1038/s41467-020-19978-1



## Intercellular and intracellular cilia orientation is coordinated by CELSR1 and CAMSAP3 in oviduct multi-ciliated cells

Usami, F.M., Arata, M., Shi, D., Oka, S., Higuchi, Y., Tissir, F., Takeichi, M., and Fujimori, T. (2021). *Journal of Cell Science* 134. DOI:10.1242/jcs.257006



## Professor Emeritus



Professor Emeritus  
TAKEUCHI, Ikuo



Professor Emeritus  
SUZUKI, Yoshiaki



Professor Emeritus  
MOHRI, Hideo



Professor Emeritus  
KATSUKI, Motoya



Professor Emeritus  
NAGAHAMA, Yoshitaka



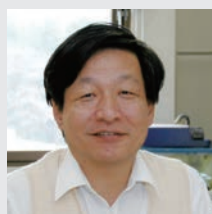
Professor Emeritus  
OHSUMI, Yoshinori



Professor Emeritus  
HORIUCHI, Takashi



Professor Emeritus  
OKADA, Kiyotaka



Professor Emeritus  
NISHIMURA, Mikio



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YAMAMORI, Tetsuo



Professor Emeritus  
IGUCHI, Taisen



Professor Emeritus  
YAMAMOTO, Masayuki



Professor Emeritus  
NODA, Masaharu

### [Original Paper by Yoshitaka Nagahama]

- Yamashita, J., Takeuchi, A., Hosono, K., Fleming, T., Nagahama, Y., and Okubo, K. (2020). Male-predominant galanin mediates androgen-dependent aggressive chases in medaka. *eLife* 9. DOI: 10.7554/eLife.59470

### [Original Paper by Mikio Nishimura]

- Stefanik, N., Bizan, J., Wilkens, A., Tarnawska-Glatt, K., Goto-Yamada, S., Strzalka, K., Nishimura, M., Hara-Nishimura, I., and Yamada, K. (2020). NAI2 and TSA1 drive differentiation of constitutive and inducible ER body formation in brassicaceae. *Plant Cell Physiol.* 61, 722–734. DOI: 10.1093/pcp/pcz236

### [Original Paper by Tetsuo Yamamori]

- Sasaki, T., Komatsu, Y., and Yamamori, T. (2020). Expression patterns of *SLIT/ROBO* mRNAs reveal a characteristic feature in the entorhinal-hippocampal area of macaque monkeys. *BMC Res. Notes* 13, 262. DOI: 10.1186/s13104-020-05100-7

### [Original Papers by Masaharu Noda]

- Matsuda, T., Hiyama, T.Y., Kobayashi, K., Kobayashi, K., and Noda, M. (2020). Distinct CCK-positive SFO neurons are involved in persistent or transient suppression of water intake. *Nat. Commun.* 11. DOI: 10.1038/s41467-020-19191-0
- Perera, S.N., Williams, R.M., Lyne, R., Stubbs, O., Buehler, D.P., Sauka-Spengler, T., Noda, M., Micklem, G., Southard-Smith, E.M., and Baker, C.V.H. (2020). Insights into olfactory ensheathing cell development from a laser-microdissection and transcriptome-profiling approach. *Glia* 68, 2550–2584. DOI: 10.1002/glia.23870

**DIVISION OF CELLULAR DYNAMICS**



Professor  
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 KANAZAWA, Takehiko

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Technical Staff: HAYASHI, Kohji  
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 FENG, Yihong

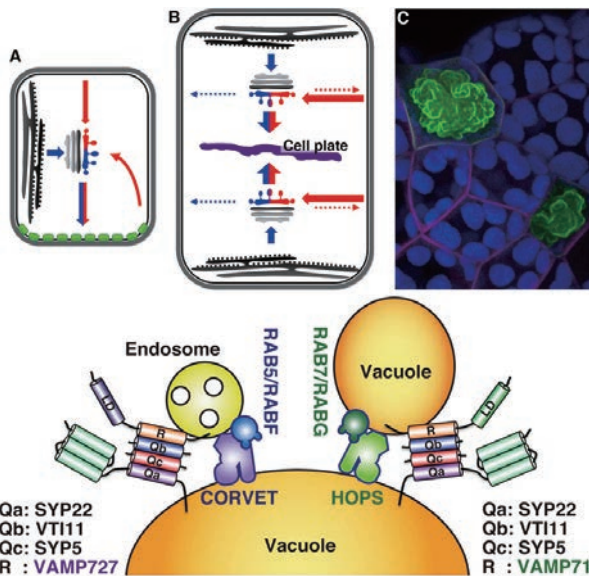
SOKENDAI Graduate Student: HACHINODA, Sho

Visiting Graduate Student: NORIZUKI, Takuya

Technical Assistant: YAMAMOTO, Mayuko  
 OHARA, Satomi

YOSHINORI, Yumi

Admin Support Staff: OKUBO, Masayo



Visual overview of this lab's work.

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.

**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 remain unclear. 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 a member of the SYP1 group. Secretory cargos were targeted to the oil body (Figure 1), which indicated 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.

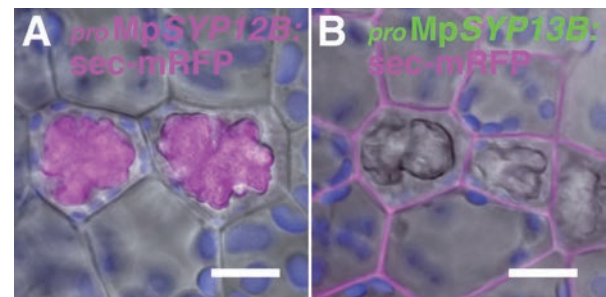


Figure 1. The redirection of the secretory pathway in oil body cells. The secretory cargo, sec-mRFP, is targeted to oil bodies (A) and the extracellular space (B), when expressed in hypothetical "oil body phase" and "plasma membrane phase," respectively. Bars = 10  $\mu$ m. (Kanazawa *et al.*, 2020)



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

### 1-2 Mechanisms and dynamics of vacuolar transport

The vacuole is the largest organelle in plant cells, and occupies over 90% of mature plant cells. The vacuole fulfills various functions in plant physiology and development, such as protein degradation, protein storage, and the regulation of turgor pressure. To perform these vacuolar functions, a wide variety of vacuolar proteins and other components must be properly transported to the vacuole, the entirety of which is mediated by membrane trafficking, which is a process distinctly regulated from non-plant systems (for example, Takemoto *et al.*, 2018).

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

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

### 2-1 Functions of ANTH-domain proteins in plant physiology

AP180 N-terminal homology domain-containing proteins (ANTH proteins) are thought to act as adaptors bridging the clathrin coat and cargo proteins during clathrin-coated vesicle formation. ANTH proteins have 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*.

Consequently, we are now investigating the molecular function of PICALMs with a special focus on the mechanisms of cargo recognition by PICALMs in *A. thaliana*.

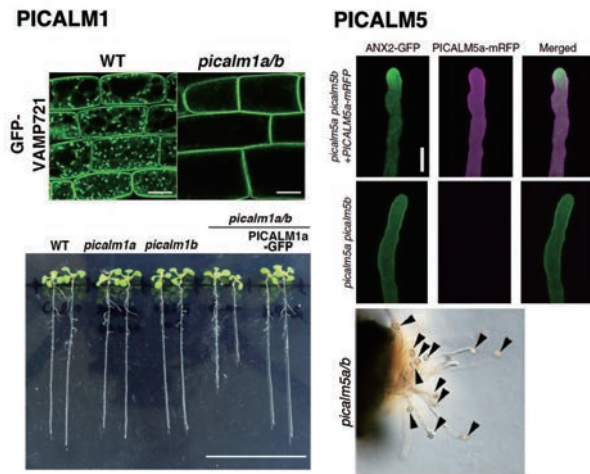


Figure 2. PICALM1 and PICALM5 mediate endocytosis of distinct plasma membrane proteins, which are required for normal vegetative development and pollen tube integrity, respectively. (Left: Fujimoto *et al.*, 2020, modified, right: Muro *et al.*, 2018, modified)

### 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*.

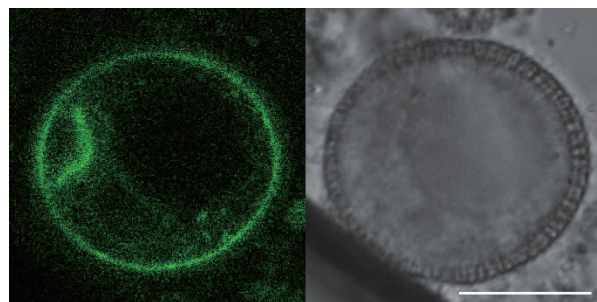


Figure 3. Expression and subcellular localization of GFP-KNOLLE during pollen mitosis I. GFP-KNOLLE accumulates at the cell plate. Bar = 10  $\mu$ m.

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 MpATG5 and MpATG7, which are key factors for autophagosome formation, affected the transportation of cytosolic components to the vacuole for degradation (Figure 4).

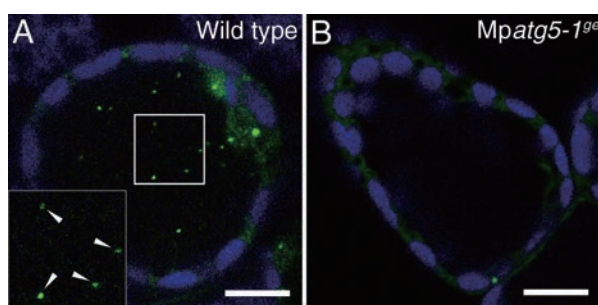


Figure 4. Establishment of analytical tools for the autophagy study in *M. polymorpha*. YFP-tagged MpATG8a is targeted to vacuole in wild type (A), but not in the *Mpatg5-1<sup>oe</sup>* mutant (B). Bars = 10  $\mu$ m. (Norizuki *et al.*, 2019).

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 persists in mature spermatozoids, which implies that there should be a mechanism for selective removal of unneeded organelles, which we are also investigating. 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).

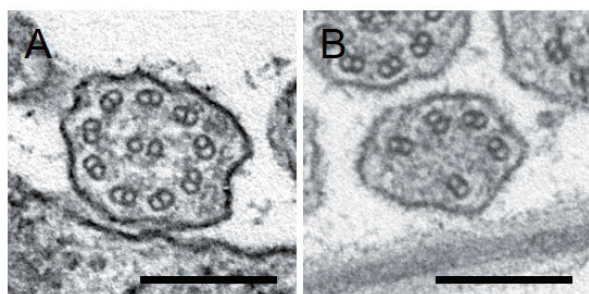


Figure 5. Transverse sections of flagella in wild-type (A) and mutant (B) spermatids. The microtubule-based “9 + 2” axoneme structure is severely compromised in the mutant. Bars = 200 nm.

## Publication List:

### [Original papers]

- Duan, Z., Tanaka, M., Kanazawa, T., Haraguchi, T., Takyu, A., Era, A., Ueda, T., Ito, K., and Tominaga, M. (2020). Characterization of ancestral myosin XI from *Marchantia polymorpha* by heterologous expression in *Arabidopsis thaliana*. *Plant J.* 104, 460–473. DOI: 10.1111/tpj.14937
- Fujimoto, M., Ebine, K., Nishimura, K., Tsutsumi, N., and Ueda, T. (2020). Longin R-SNARE is retrieved from the plasma membrane by ANTH domain-containing proteins in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 117, 25150–25158. DOI: 10.1073/pnas.2011152117
- Kanazawa, T., Morinaka, H., Ebine, K., Shimada, T.L., Ishida, S., Minamino, N., Yamaguchi, K., Shigenobu, S., Kohchi, T., Nakano, A., Ueda, T. (2020). The liverwort oil body is formed by redirection of the secretory pathway. *Nat. Commun.* 11. DOI: 10.1038/s41467-020-19978-1
- Lupanga, U., Roehrich, R., Askani, J., Hilmer, S., Kiefer, C., Krebs, M., Kanazawa, T., Ueda, T., and Schumacher, K. (2020). The *Arabidopsis* V-ATPase is localized to the TGN/EE via a seed plant-specific motif. *eLife* 9. DOI: 10.7554/eLife.60568
- Otsuka, K., Mamiya, A., Konishi, M., Nozaki, M., Kinoshita, A., Tamaki, H., Arita, M., Saito, M., Yamamoto, K., Hachiya, T., *et al.* (2021). Temperature-dependent fasciation mutants provide a link between mitochondrial RNA processing and lateral root morphogenesis. *eLife* 10. DOI: 10.7554/eLife.61611
- Romani, F., Banic, E., Florent, S.N., Kanazawa, T., Goodger, J.Q.D., Mentink, R.A., Dierschke, T., Zachgo, S., Ueda, T., Bowman, L.J., Tsiantis, M., *et al.* (2020). Oil body formation in *Marchantia polymorpha* is controlled by MpC1HDZ and serves as a defense against arthropod herbivores. *Curr. Biol.* 30, 2815+. DOI: 10.1016/j.cub.2020.05.081
- 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. DOI: 10.1080/15592324.2021.1872217
- Shimizu, Y., Takagi, J., Ito, E., Ito, Y., Ebine, K., Komatsu, Y., Goto, Y., Sato, M., Toyooka, K., Ueda, T., *et al.* (2021). Cargo sorting zones in the trans-Golgi network visualized by super-resolution confocal live imaging microscopy in plants. *Nat. Commun.* 12. DOI: 10.1038/s41467-021-22267-0
- Suzuki, R., Ueda, T., Wada, T., Ito, M., Ishida, T., and Sawa, S. (2021). Identification of genes involved in *Meloidogyne incognita*-induced gall formation processes in *Arabidopsis thaliana*. *Plant Biotech.* 38. DOI:10.5511/plantbiotechnology.20.0716a

### [Review article]

- Norizuki, T., Minamino, N., and Ueda, T. (2020). Role of autophagy in male reproductive processes in land plants. *Front. Plant Sci.* 11. DOI: 10.3389/fpls.2020.00756

### [Book Chapter]

- Ito, E., Choi, S.W., and Ueda, T. (2020). Purification and interaction analysis of a plant-specific RAB5 effector by in vitro pull-down assay. *Methods Mol Biol* 2177, 183-197. DOI: 10.1007/978-1-0716-0767-1\_15

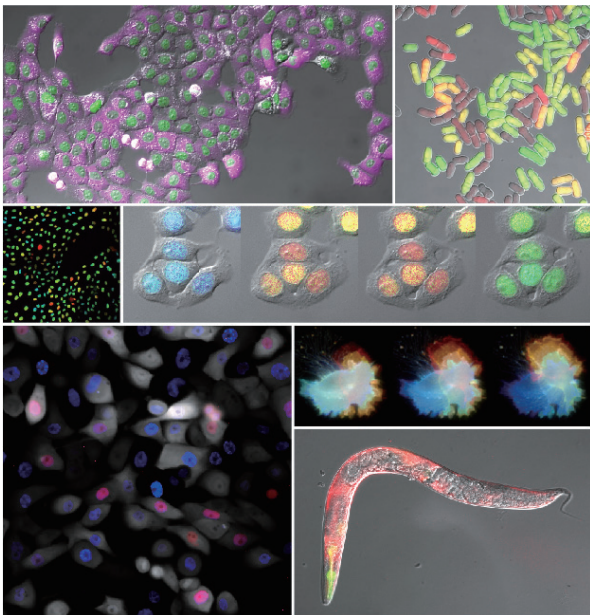
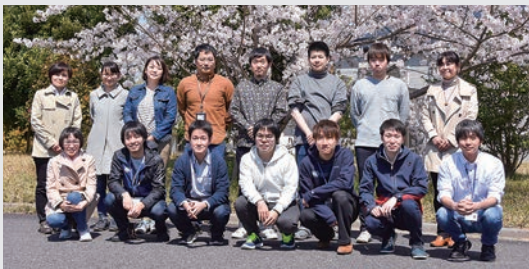


**DIVISION OF QUANTITATIVE BIOLOGY**



Professor  
**AOKI, Kazuhiro**

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 GOTO, Yuhei
- Technical Staff:** BINO, Takahiro
- Postdoctoral Fellow:** NAKAMURA, Akinobu
- SOKENDAI Graduate Student:** TANII, Ryosuke  
 MUKAI, Masaya  
 YAMAMOTO, Kei  
 SAKAI, Keiichiro  
 TSURUOKA, Tatsuki
- Visiting Scientist:** ITO, Reina
- Technical Assistant:** EBINE, Emi  
 GOTO, Yoko
- 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).

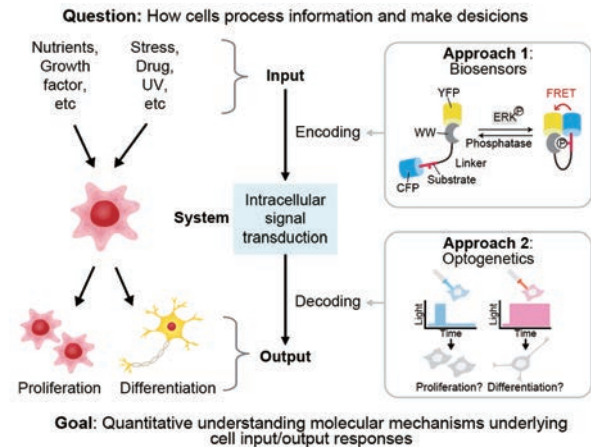


Figure 1. Information processing by intracellular signaling devices and networks.

**I. Visualization of cell signaling**

With the advent of fluorescent proteins, it has become possible to visualize kinetic reactions at a 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). By using a FRET biosensor, we have been able to reveal the role played by ERK activation dynamics in cell proliferation (Aoki K, Mol Cell, 2013) and collective cell migration (Aoki K, Dev Cell, 2017).

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-permuted mApple (cpmApple), a red fluorescent protein, and a cpmApple is inserted into the third intracellular loop of a DA receptor, DRD1 (Figure 2A). The linker sequences between DRD and cpmApple were optimized by random mutagenesis and subsequent live-cell screening. Finally, we picked up the best performance reporter and named the red genetically encoded

GPCR activation reporter for DA, R-GenGAR-DA. The red fluorescence intensity decreased in response to DA and returned to the basal level upon a DRD1 antagonist treatment (Figure 2B and 2C). In addition, by using a previously reported green fluorescence norepinephrine (NE) reporter, we succeeded in simultaneously visualizing DA and NE in living neurons (Nakamoto C, Goto Y, *et al.*, bioRxiv, 2020).

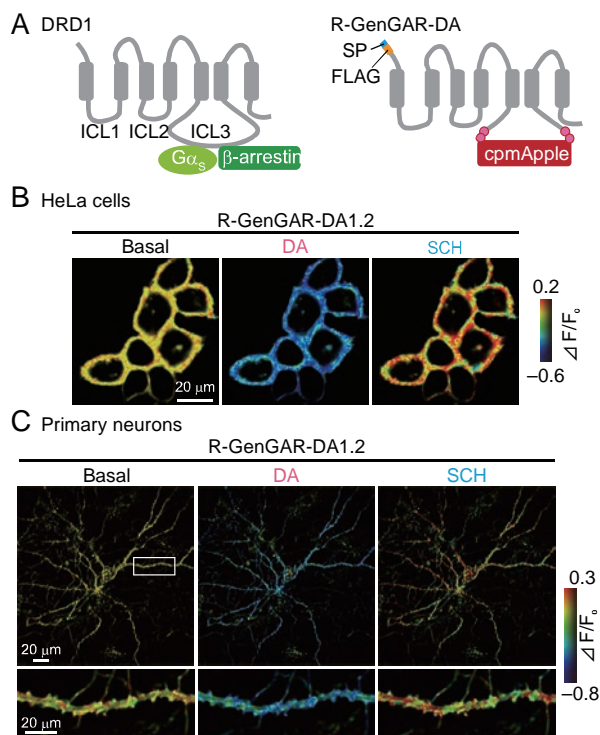


Figure 2. Development of a genetically encoded red fluorescent dopamine (DA) reporter. (A) Schematic representation of a dopamine receptor, DRD1 (left), and Red DA reporter, R-GenGAR-DA. (B and C) HeLa cells (B) or mouse primary hippocampal neurons (C) expressing R-GenGAR-DA were stimulated with DA,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

## 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 controlling 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 3A). We confirmed that blue-light illumination was sufficient to induce dephosphorylation of MLC, resulting in lamellipodial membrane protrusion (Figure 3B). The OptoMYPT was further employed to understand the mechanics of actomyosin-based cortical tension and contractile ring tension during cytokinesis (Figure 3C). We found that the relaxation of cortical tension at both poles by OptoMYPT accelerated 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, *et al.*, bioRxiv, 2021).

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 are focusing on the phytochrome B (PhyB)-PIF LID system. PhyB binds to PIF upon red-light illumination, and the PhyB-PIF complex dissociates from each other by far-red light exposure (Figure 4A). The

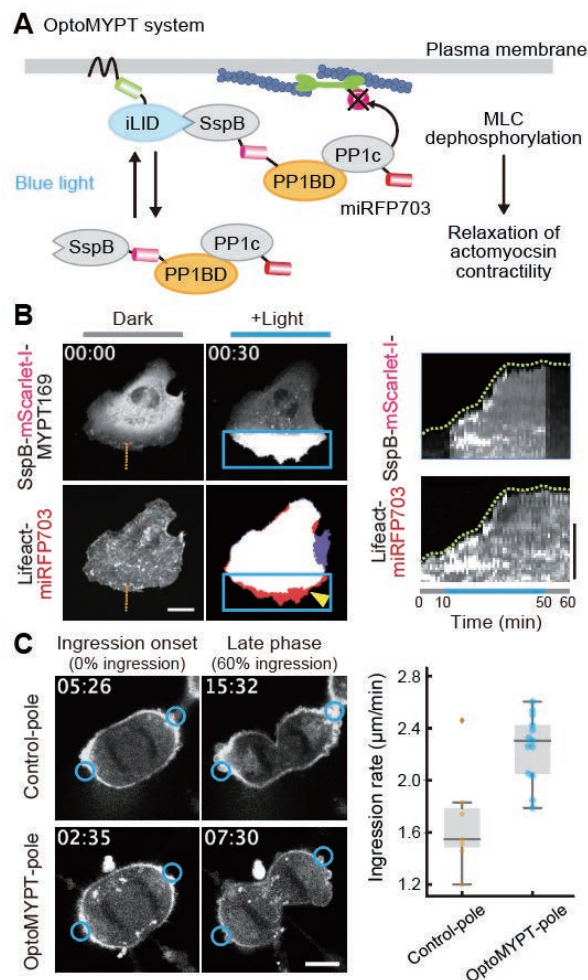


Figure 3. OptoMYPT: Optogenetic relaxation of actomyosin contractility. (A) Schematic representation of OptoMYPT. (B) Local recruitment of OptoMYPT induced myosin regulatory light chain dephosphorylation, leading to the induction of membrane protrusion. (C) Local relaxation of actomyosin cortical tension at the both poles in mitotic cells accelerated ingression of contractile ring.



reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system because other LID systems can only control association or dissociation by light. One drawback is that covalent attachment of a chromophore, phycocyanobilin (PCB), is required for the light-responsive function of PhyB-PIF. To overcome this issue, we have developed a method for PCB biosynthesis in mammalian cells by introducing the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into the mitochondria (Uda Y, *et al.*, PNAS, 2017) (Figure 4A). We recently improved the PCB synthesis system, named SynPCB2.1, which allowed establish-

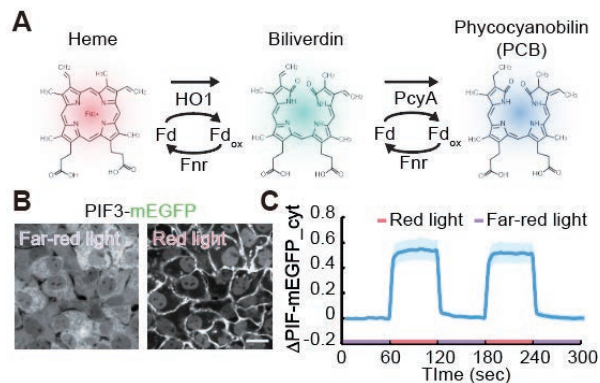


Figure 4. PhyB-PIF light-inducible dimerization (LID) system. (A) The metabolic pathway of phytochrome chromophores, PCB. (B and C) Light-induced translocation of PIF3-mEGFP to the plasma membrane, where PhyB is localized (B). Membrane translocation of PIF3-mEGFP is quantified (C).

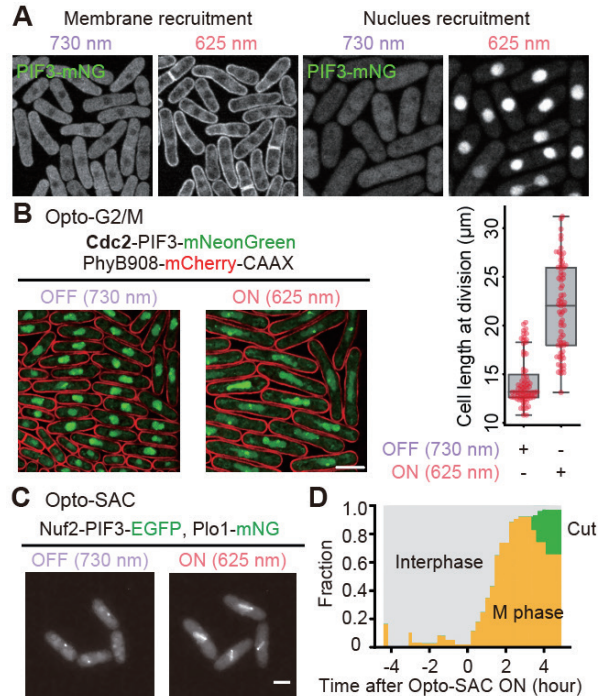


Figure 5. Optogenetic control of cell cycle in fission yeast. (A) Light-induced recruitment of PIF3-mNeonGreen (mNG) to the plasma membrane (left) and nucleus (right). (B) Opto-G2/M: Optical control of G2/M transition. (left) Opto-G2/M OFF cells showed normal morphology, while Opto-G2/M ON cells exhibited 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.

ing stable cell lines that synthesize PCB and light-induced control of protein localization at the cell population level (Figure 4B and 4C) (Uda Y, *et al.*, ACS Chem Biol, 2020).

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

## Publication List:

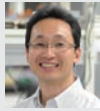
### [Original papers]

- Asakura, Y., Kondo, Y., Aoki, K., and Naoki, H. (2021). Hierarchical modeling of mechano-chemical dynamics of epithelial sheets across cells and tissue. *Sci. Rep. 11*. DOI: 10.1038/s41598-021-83396-6
- Hino, N., Rossetti, L., Marin-Llaurado, A., Aoki, K., Trepas, X., Matsuda, M., and Hirashima, T. (2020). ERK-mediated mechanochemical waves direct collective cell polarization. *Dev. Cell 53*, 646+. DOI: 10.1016/j.devcel.2020.05.011
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- Akiyama, S., Aoki, K., and Kubo, Y. (2020). Biophysical research in Okazaki, Japan. *Biophys. Rev. 12*, 237–243. DOI: 10.1007/s12551-020-00633-4
- Goto Y., Kondo Y., Aoki K. (2021). Visualization and Manipulation of Intracellular Signaling. *Adv. Exp. Med. Biol. 1293*, 225–234. DOI: 10.1007/978-981-15-8763-4\_13

## DIVISION OF CHROMATIN REGULATION



Professor  
NAKAYAMA, Jun-ichi

Assistant Professor: KATAOKA, Kensuke  
Specially Appointed Assistant Professor:

HAYASHI, Aki

Technical Staff: NISHIMOTO, Yuki

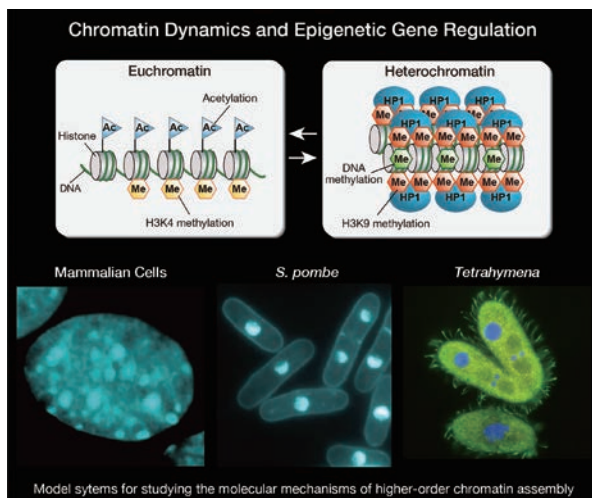
SOKENDAI Graduate Student: RAHAYU, Anisa  
VALENTIROVIC, Olivera  
NAKAMURA, Rinko  
YOSHIDA, Takaki

Visiting Graduate Student: HACHISUKA, Aki

Visiting Scientist: HAMADA, Kyoko

Technical Assistant: YOSHIMURA, Yuriko  
ASAI, Yuriko

Admin Support Staff: KIYOHARA, Megumi



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

We are also attempting to determine the cellular functions of chromatin modifying factors so that we can develop a better understanding of complicated epigenetic phenomena in higher eukaryotic cells.

## I. Establishment and maintenance of higher-order chromatin structures

### 1-1 Mechanisms regulating Clr4 histone methyltransferase activity

In eukaryotic cells, the assembly of higher-order chromatin structures, known as 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. 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. 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 can potentially interact with the SET domain and that this interaction requires both CD and its adjacent region (Figure 1B). To determine the regions/residues responsible for this interaction, we performed cross-linking mass spectrometry analyses and found that crosslinked residues were concentrated in a region near the C-terminal end of the SET domain (Figure 1C). These results support the idea that Clr4's enzymatic activity is regulated by inter- or intra-domain interactions.

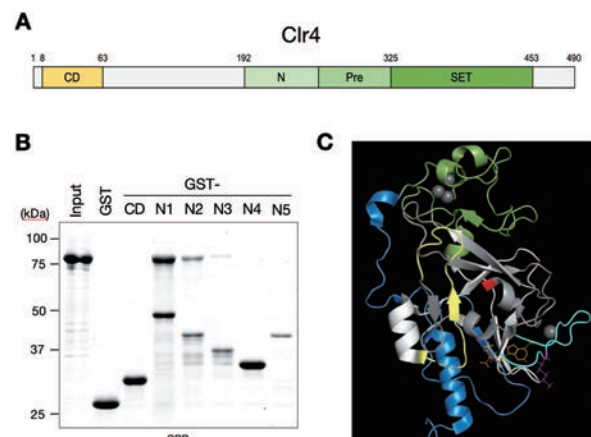


Figure 1. Characterization of inter- and/or intra-domain interactions of Clr4. (A) Domain organization of Clr4. The N-terminal chromodomain (CD) recognizes H3K9me, and the C-terminal SET domain is responsible for Clr4's enzymatic activity. (B) Pull-down assays of recombinant Clr4 to determine domains required for the interaction. Proteins were analyzed by SDS-PAGE and CBB 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.

## 1-2 Characterization of Clr4 methyltransferase complex (CLRC) in fission yeast

In fission yeast, Clr4 is the sole H3K9 methyltransferase and is essential for heterochromatin assembly. Biochemical and genetic analyses revealed that Clr4 forms a multiprotein complex called CLRC. CLRC consists of Cul4, Rik1, Raf1, Raf2, and Rbx1, and shows structural similarity to the CUL4-DDB1 complex involved in the repair of damaged DNA. We previously have demonstrated that affinity-purified CLRC ubiquitylates histone H3 and that H3 lysine 14 (H3K14) is the preferred target of the complex. We further demonstrated that CLRC-mediated H3 ubiquitylation promotes H3K9me by Clr4, suggesting a cross-talk mechanism between histone ubiquitylation and methylation that is involved in heterochromatin assembly (Oya *et al.* 2019). Although this study uncovered one of the physiological targets of CLRC, how Clr4 interacts with other components in CLRC remains unclear. To investigate the functional relationship between Clr4 and the CLRC, we expressed FLAG-tagged Clr4 in a strain expressing Rik1-myc and performed an IP-western experiment to confirm the interaction between Clr4 and Rik1 (CLRC). Using this assay, we expressed a series of Clr4 deletion mutants and successfully identified a domain which is necessary for the interaction with CLRC. Based on the above results, we performed a yeast two-hybrid assay and identified a candidate CLRC component responsible for the binding to Clr4. These results suggest that the interaction between Clr4 and CLRC is coupled with Clr4's intramolecular conformational change.

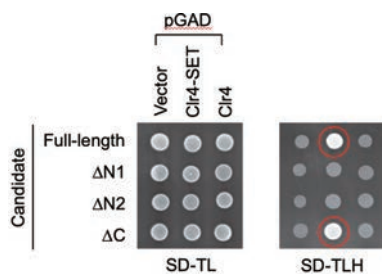


Figure 2. A representative result of Yeast two-hybrid assay using strains expressing full-length Clr4 or Clr4-SET in combination with full-length or truncated mutant of the candidate protein.

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

### 2-1 Mitotic phosphorylation of Swi6/HP1 regulates mitotic chromosome segregation

HP1 is a conserved chromosomal protein that plays important roles in heterochromatin assembly. There is growing evidence that HP1-family proteins undergo a variety of post-translational modifications, including phosphorylation, acetylation, methylation, and ubiquitination. Phosphorylation, the most extensively studied of these modifications, is widely implicated in HP1's dynamics and functions. We previously showed that Swi6, one of two HP1 isoforms in *S. pombe*, is constitutively phosphorylated by casein kinase II (CK2) and this phosphorylation is essential for its function in heterochromatin assembly. Several previous studies demonstrated that HP1 is subject to additional phosphoryla-

tion during mitosis. However, the functional importance of HP1's mitotic phosphorylation remains unclear. Using *E. coli* co-expression system, we demonstrated that Swi6 is phosphorylated by Ark1, a solo Aurora kinase in *S. pombe*. Subsequent mutation analyses revealed that serine residues in the N-terminal region of Swi6 are efficient targets for Ark1. We confirmed that these serine residues are phosphorylated during mitosis *in vivo*. Interestingly, expression of mutant Swi6 containing amino-acid substitutions at the serine residues differentially modulates temperature-sensitive growth of the mutations for Chromosome Passenger Complex (CPC) components. These results suggested that Ark1-mediated Swi6 phosphorylation regulates CPC's function during mitosis.

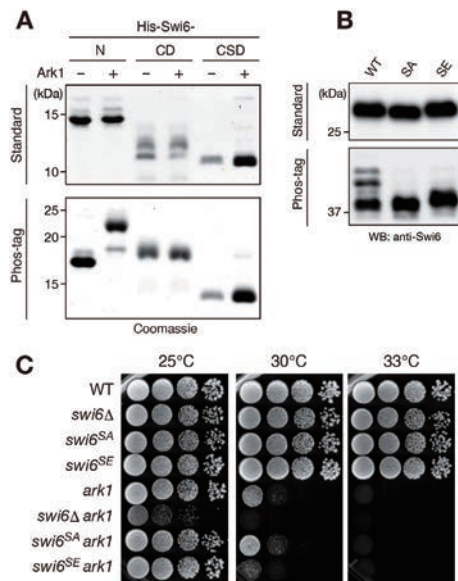


Figure 3. Mitotic phosphorylation of Swi6 regulates mitotic chromosome segregation. (A) Swi6 domains, N-terminal region (N), CD, and CSD, produced in control or control or Ark1-co-expressed *E. coli* cells were purified, resolved by standard or Phos-tag PAGE, and visualized by CBB staining. (B) Phosphorylation patterns of wild-type (wt) or mutant Swi6 proteins were analyzed by immunoblotting using anti-Swi6 antibody. (C) Serially diluted cells of the indicated strains were spotted on non-selective media and incubated at indicated temperatures.

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

### 3-1 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 includ-



ing Pdd1 induced the elimination of the tethered site (Figure 5). 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.

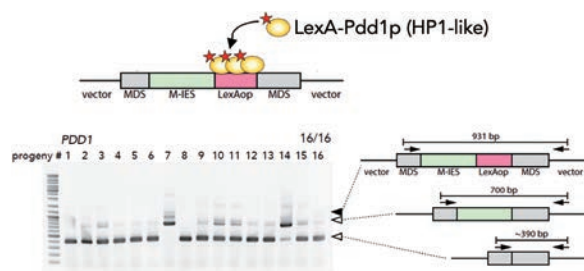


Figure 4. Identification of heterochromatin proteins sufficient to induce DNA elimination. Schematics of the tethering assay (top). A representative result of the tethering assay using Pdd1p (bottom).

### 3-2 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 5). 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 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 H3K9/27me marks over transposon loci and facilitates the heterochromatin formation by interacting with heterochromatin components required for DNA

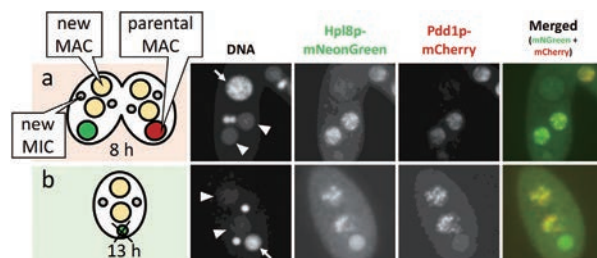


Figure 5. Cells expressing the Hpl8-mNeonGreen (green) and Pdd1p-mCherry (red) at 8 and 13 hours post induction of mating are shown in (a) and (b), respectively. DNA was counterstained with DAPI. Arrows and arrowheads indicate the parental and new MACs, respectively.

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.

## IV. 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. We isolated fission yeast spores by density-gradient centrifugation and performed mass spectrometry analysis to determine spore-specific nuclear proteins. 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. These results imply that dynamic changes in active histone demonstrate a contribution to spore formation and epigenetic inheritance.

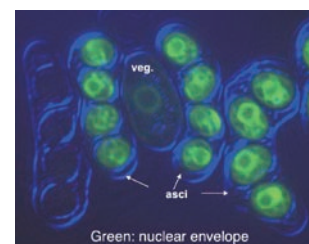


Figure 6. A representative image of vegetative cells (veg.) and spores in asci.

### Publication List:

#### [Original paper]

- Dong, C., Nakagawa, R., Oyama, K., Yamamoto Y., Zhang, W., Dong, A., Li, Y., Yoshimura Yuriko and Kamiya, H., Nakayama, J., Ueda, J., and Min, J. (2020). Structural basis for histone variant H3tK27me3 recognition by PHF1 and PHF19. *eLife* 9, e58675. DOI: 10.7554/eLife.58675

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- Hiragami-Hamada, K., Tani, T., and Nakayama, J. (2020). Proteomics-based systematic identification of nuclear proteins anchored to chromatin via RNA. *Methods Mol Biol* 2161, 88-99. DOI: 10.1007/978-1-0716-0680-3\_8

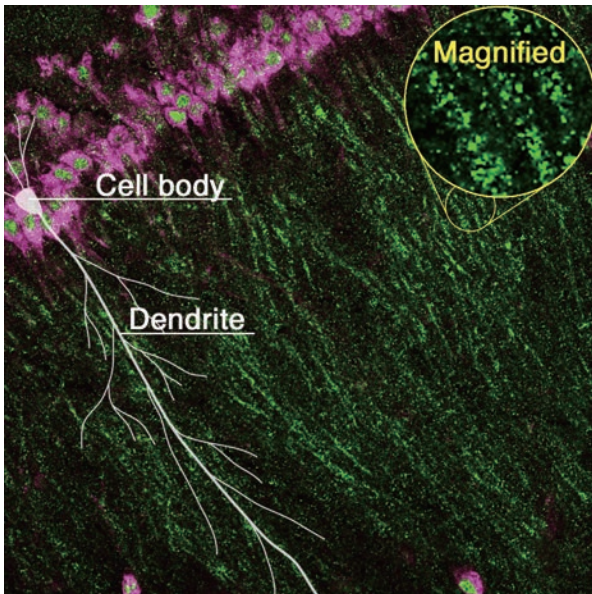


**LABORATORY OF NEURONAL CELL BIOLOGY**



Associate Professor  
 SHIINA, Nobuyuki

Assistant Professor: NAKAYAMA, Kei  
 OHASHI, Rie  
 SOKENDAI Graduate Student: YAMASHITA, Akira  
 HORIO, Tomoyo  
 ISHIKURA, Yui  
 YOSHIDA, Sho  
 Technical Assistant: KATAYAMA, Kaori



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

The transportation 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 stimula-

tion-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 incorporation 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.

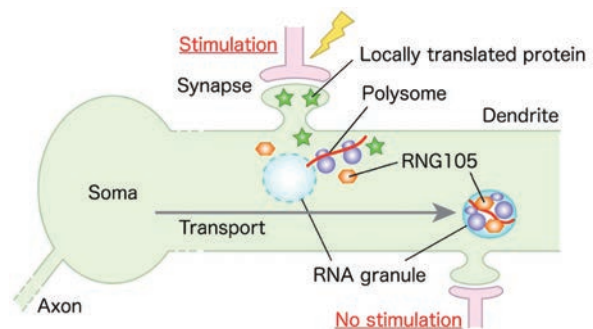


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

**I. Stress tolerance of conditioned fear memory conferred by alternatively spliced prion-like domain of *Ilf3***

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 well studied in regards to FUS and TDP-43 in 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. Therefore, it is possible to investigate the role specific to PrLD by deleting PrLD of NFAR2, since NFAR1 without PrLD is intrinsically present and maintained even after the deletion of PrLD. 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 associate with cytoplasmic RNA granules via PrLD. To investigate the physiological relevance of PrLD, we generated NFAR2ΔPrLD mice, in which a stop codon was introduced into the NFAR2-specific exon.

Deletion of the PrLD lost the nucleoplasmic localization of NFAR2 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 its effect on CRS-induced changes in mRNA expression and translation, PrLD deletion affected tolerance of amygdala-associated learning and memory to CRS: conditioned fear memory in wild-type mice was unaffected by CRS, but it was exacerbated in NFAR2ΔPrLD mice due to this factor. These results suggested that PrLD of NFAR2 conferred tolerance of fear-associated memory formation to stressful environments.

## II. Incorporation of FUS and TDP-43 into RNA granules releases RNG105 (Caprin1) from the granules

FUS and TDP-43 are predominantly localized in the nucleus when the cell is healthy, but translocate to the cytoplasm and incorporate into RNA granules when stricken by neurodegenerative diseases. This incorporation is thought to affect the fluidity of the components of RNA granules formed by LLPS, but little is known about their effects. We overexpressed either disease-related FUS mutants or disease related TDP-43 mutants in primary cultured neurons from the mouse cerebral cortex and analyzed their effects on the fluidity 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 fluidity of FMRP or PUM2 in dendritic RNA granules, but did affect the fluidity of RNG105 (Caprin1) (Figure 2). The fluidity of RNG105 was significantly increased, resulting in the release of RNG105 from RNA granules into the cytoplasm of dendrites. Since RNG105 is required for mRNA transport to dendrites and formation of long-term memory (Nakayama *et al.*, *eLife*, 2017), loss of RNG105 in dendritic RNA granules may affect these functions. We are currently investigating the molecular mechanisms underlying the changes in fluidity specific to RNG105 and the effects of FUS and TDP-43 incorporation into RNA granules on mRNA recruitment into RNA

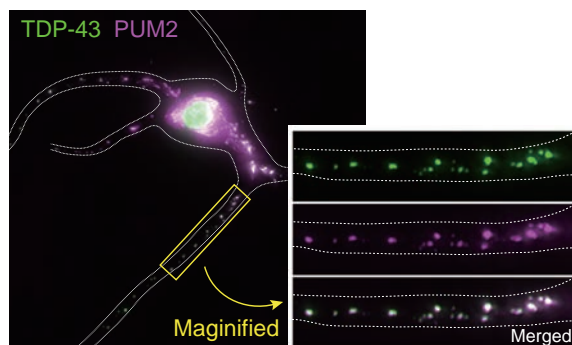


Figure 2. Incorporation of neurodegenerative disease-related proteins into RNA granules. Primary cultured neurons from mouse cerebral cortex were transfected with disease-related proteins (TDP-43-GFP in this case) together with RNA granule components (PUM2-mRFP1 in this case). The fluidity of these proteins in RNA granules was measured by FRAP and cell permeabilization assays.

granules, local translation in the vicinity of RNA granules, and synapse formation in dendrites.

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

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

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

RNG140-mediated translational regulation also operates in the mouse eye, where RNG140 knockout increased the translation of long mRNAs. mRNAs involved in lens differentiation, such as crystallin mRNAs, are short, and were able to escape translational inhibition by RNG140 and be translated in differentiating lenses (Figure 3). These findings provide insight into the mechanistic basis of lens cell transition from proliferation to differentiation via RNG140-mediated translational regulation. Moreover, the preference for long mRNAs raised new questions about why and how RNA-binding protein complexes distinguish mRNA lengths in the coordination of proliferation and differentiation.

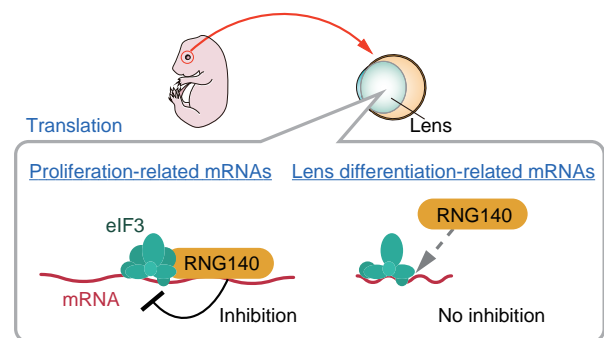


Figure 3. mRNA length-selective inhibition of translation by RNG140 in eye lens differentiation.

### 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., *et al.* (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

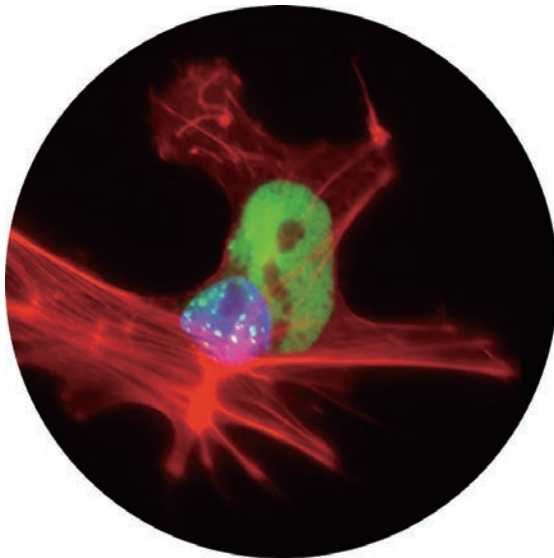


Associate Professor  
TSUBOUCHI, Tomomi

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



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



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”). Pluripotent cells exist only transiently and are lost as development proceeds. However, ES cells are capable of proliferating indefinitely when given an appropriate culturing condition. Curiously, ES cells proliferate with truncated gap phases while lengths of S (DNA replication) and M (mitosis) phases are similar to other cell types. ES cells also appear to lack some of the mechanisms that ensure genome integrity (*i.e.*, checkpoint mechanisms). The significance of this phenomenon remains a mystery.

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

### II. Genome Instability during Nuclear Reprogramming

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

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

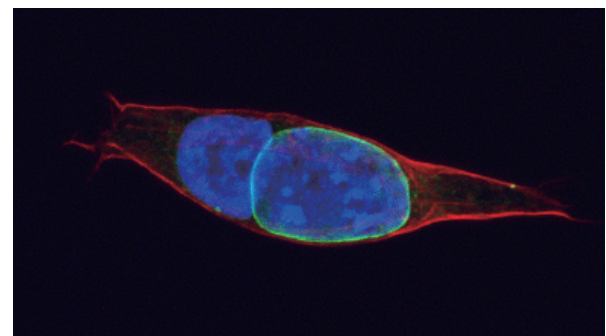


Figure 1. Cellular fusion to study reprogramming: a human lymphoblastoid nucleus can be induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green). Lamin B1 is endogenously tagged with GFP in ES cells, allowing us to distinguish ES vs lymphoblastoid nucleus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

Using this system, we previously found that DNA synthesis is an important event for successful reprogramming (Tsubouchi *et al.*, Cell, 2013). Recent reports indicate that



reprogramming may cause genetic instabilities, some of which are thought to arise as DNA replication errors. To investigate the nature of such errors and how they are linked to reprogramming-specific events, we are in the process of setting up a system to isolate and track a single fused cell through live-imaging (Figure 1).

### III. Future Perspectives

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

#### Publication List:

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[Original paper]

- Kobayashi, M.I., Angelone, M., Yoshihashi, S., Ogawa, K., Isobe, M., Nishitani, T., Sangaroon, S., Kamio, S., Fujiwara, Y., Tsubouchi T., Uritani, A., *et al.* (2020). Thermal neutron measurement by single crystal CVD diamond detector applied with the pulse shape discrimination during deuterium plasma experiment in LHD. *Fusion Eng. Des.* 161. DOI: 10.1016/j.fusengdes.2020.112063

## LABORATORY OF ORGANELLE REGULATION



Associate Professor  
MANO, Shoji

Specially Appointed Assistant Professor:

KANAI, Masatake

Visiting Scientist:

KAMIGAKI, Akane

Technical Assistant:

HIKINO, Kazumi

NAGATA, Kyoko

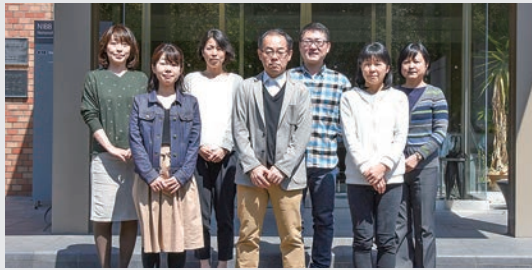
NAKAYAMA, Tomomi

HOSHI, Rie

Admin Support Staff:

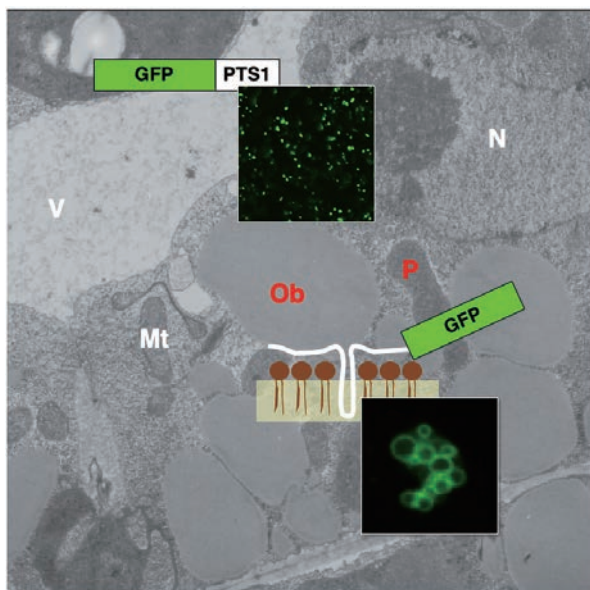
UEDA, Chizuru

ASAI, Sanae



Because plants spread their roots in the ground, they must survive in a given environment. To adapt to their environment, they interpret 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, showing plant organelles. Electronmicrograph represents Arabidopsis cotyledonary cells. Top and lower insets show the GFP-labeled peroxisomes and oil bodies, respectively. Peroxisomes and oil bodies are visualized with the fusion protein of GFP and peroxisome targeting signal 1 (PTS1), and oil body membrane protein, oleosin, respectively. Mt: mitochondrion, N; nucleus, Ob; oil body, P; peroxisome, V; vacuole.

## 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 glyoxyssomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via  $\beta$ -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* and *peup4* mutants, which were defective in Autophagy-related 2 (ATG2), ATG18a and ATG7, respectively.

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.

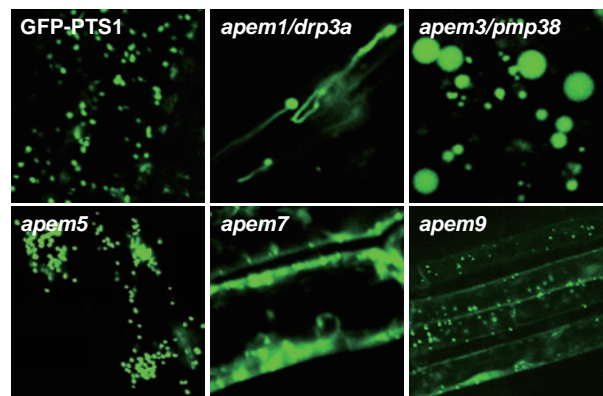


Figure 1. Phenotype of Arabidopsis *apem* mutants. GFP fluorescence was observed in the parent plant, GFP-PTS1, *apem1*, *apem3*, *apem5*, *apem7* and *apem9* mutants. *apem1* and *apem3* have elongated and enlarged peroxisomes, respectively. In *apem5* mutant, peroxisomes are aggregated. In *apem7* and *apem9*, GFP fluorescence is observed in the cytosol because of the decrease of the efficiency of protein transport to peroxisomes.

## II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves in food and industrial materials. Storage reserves vary among different types of plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybeans' 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.

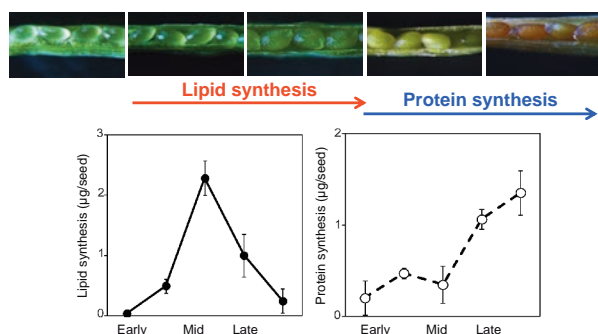


Figure 2. Biosynthesis of lipids and proteins during *Arabidopsis* seed development. Lipid synthesis and accumulation begins before those of seed storage proteins during seed development.

## 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 organelleome 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 organelleome 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.

| Thumbnail | Title of Movie, Species, Organelles   |
|-----------|---|
|           | <b>Title:</b> <i>ER bodies in Arabidopsis cotyledon</i><br><b>Species:</b> <i>Arabidopsis thaliana</i><br><b>Stimulus:</b><br><b>Organelles:</b> Endoplasmic reticulum  |
|           | <b>Title:</b> <i>Interaction of organelles</i><br><b>Species:</b> <i>Arabidopsis thaliana</i><br><b>Stimulus:</b><br><b>Organelles:</b> Peroxisome Mitochondrion Plastid  |
|           | <b>Title:</b> <i>Nuclear positioning in plant cells: an actin-regulated process</i><br><b>Species:</b> <i>Nicotiana tabacum</i> L. cv. Bright Yellow 2; BY-GF11<br><b>Stimulus:</b><br><b>Organelles:</b> Microfilament |
|           | <b>Title:</b> <i>Actin dynamics in plant cell division</i><br><b>Species:</b> <i>Nicotiana tabacum</i> L. cv. Bright Yellow 2; BY-GF11<br><b>Stimulus:</b><br><b>Organelles:</b> Microfilament                          |
|           | <b>Title:</b> <i>Actin dynamics in phragmoplast</i><br><b>Species:</b> <i>Nicotiana tabacum</i> L. cv. Bright Yellow 2; BY-GF11<br><b>Stimulus:</b>   |

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



**DIVISION OF MORPHOGENESIS**



Professor  
**UENO, Naoto**



Associate Professor  
**KINOSHITA, Noriyuki**

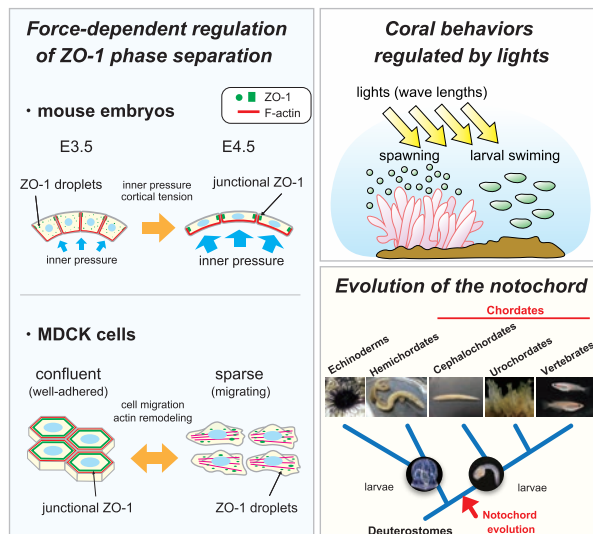
Assistant Professor:  
 Technical Staff:  
 Technical Assistant:

**TAKAHASHI, Hiroki**  
**TAKAGI, Chiyo**  
**YAMAMOTO, Takamasa**  
**YASUE, Naoko**  
**MIYAKE, Satoko**  
**TSUGE, Toyoko**

Admin Support Staff:



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 trophoblast 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



A graphical overview of our laboratory's works.

place in the sea in which to live. We also investigate the evolution of the notochord using ancestral chordates.

**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 trophoblast (TE) cells expanded and became thinner. The number of cytoplasmic ZO-1 puncta was reduced, and ZO-1 signal intensity at the plasma membrane in E4.5 embryos became much higher

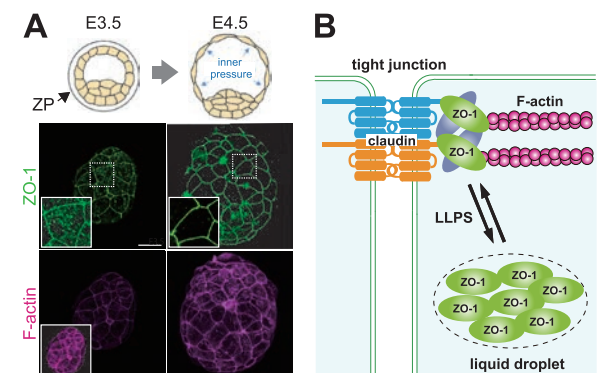


Figure 1. Change of ZO-1 localization in mouse hatching embryos. A. At E3.5, the embryo is covered with the zona pellucida (ZP). During hatching, the embryo is enlarged and emerges from the ZP. Embryos were stained with an anti-ZO-1 antibody and Alexa Fluor 546 Phalloidin. Scale bar, 20  $\mu$ m. The 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.



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<sup>+</sup>/K<sup>+</sup> 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 (refer Figure 2A), 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.

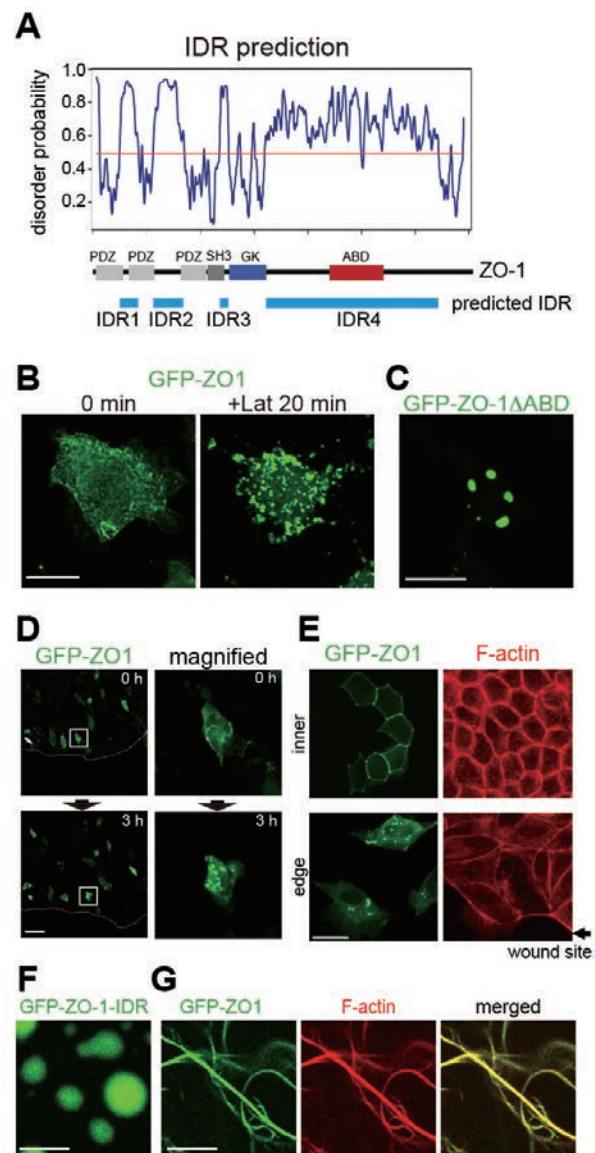


Figure 2. Interaction with F-actin regulates ZO-1 phase separation. A. The domain structure and the intrinsically disordered regions (IDRs) of ZO-1. B. A6 cells were treated with latrunculin B (Lat) to disrupt F-actin. Bar = 20 μm. C. GFP-ZO-1 lacking the actin binding domain (ABD) was expressed in A6 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.

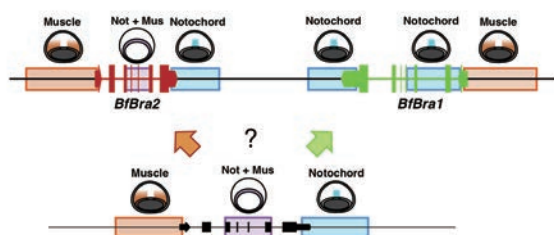


Figure 3. A summary of results of the present reporter assay and their possible interpretation.

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.

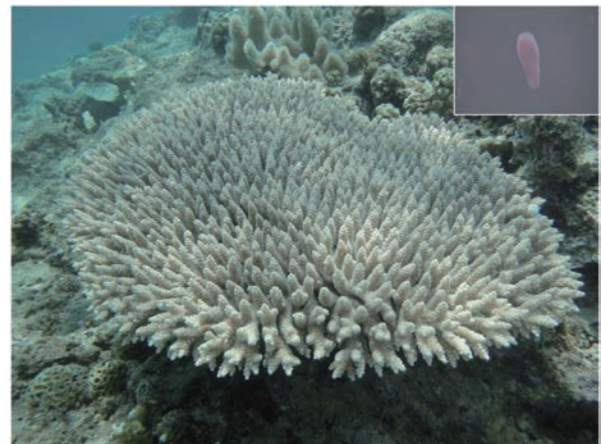


Figure 4. A wild colony of *Acropora tenuis* (photo taken by Masayuki Hatta at Ochanomizu University). The inset shows a planula larvae of *A. tenuis*.

#### Publication List:

[Original paper]

- Sakai, Y., Kato, K., Koyama, H., Kuba, A., Takahashi, H., Fujimori, T., Hatta, M., Negri, A.P., Baird, A.H., and Ueno, N. (2020). A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, *Acropora tenuis*. *Sci. Rep.* 10. DOI: 10.1038/s41598-020-74649-x

**DIVISION OF MOLECULAR AND DEVELOPMENTAL BIOLOGY**



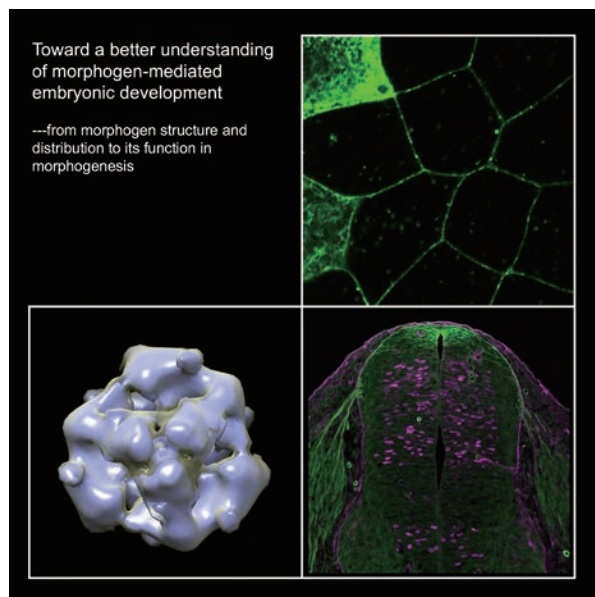
Professor  
**TAKADA, Shinji**

- Assistant Professor: **YABE, Taijiro**  
**MIJ, 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**
- Admin Support Staff: **NOBATA, Ryuko**



The morphology of the body and tissues is established in a spatio-temporally 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.

Secreted signal molecules are important in forming spatial information during the development of many tissues. These



Visual overview of this lab's work.

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.

**I. Regulation of spatial distribution of Wnt proteins in vertebrate embryos**

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

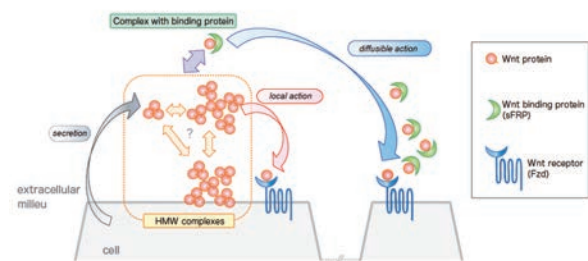


Figure 1. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion 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).



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).

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.

Firstly, we visualized their localization in *Xenopus* embryos by fusing them with fluorescent proteins. Monomeric Venus (mV) fused with Wnt8 and Frzb were visualized on the cell surface when they were expressed in *Xenopus* embryos. By contrast, we found that only the secreted form of mV (sec-mV), which was expected to have no specific binding to the cell surface, was hardly visible along the cell boundary under the same conditions. This difference in protein distribution can be explained by difference of affinity to docking molecules on the cell surface because the addition of heparin binding peptides resulted in a significant increase of the localization of sec-mV on the cell surface.

We subsequently examined the dispersion of these proteins by expressing an artificial protein, called morphotrap, that can capture Venus-tagged proteins on the cell surface (Figure 2). Because mV-Wnt8 and mV-Frzb were trapped by morphotrap, a population of mV-tagged Wnt8 and Frzb deemed not likely to associate tightly with the cell surface, and were able to be dispersed over a long range from the source cells.

We then 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. 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 the distribution and dispersion of secreted proteins. 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.

## 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 peri-

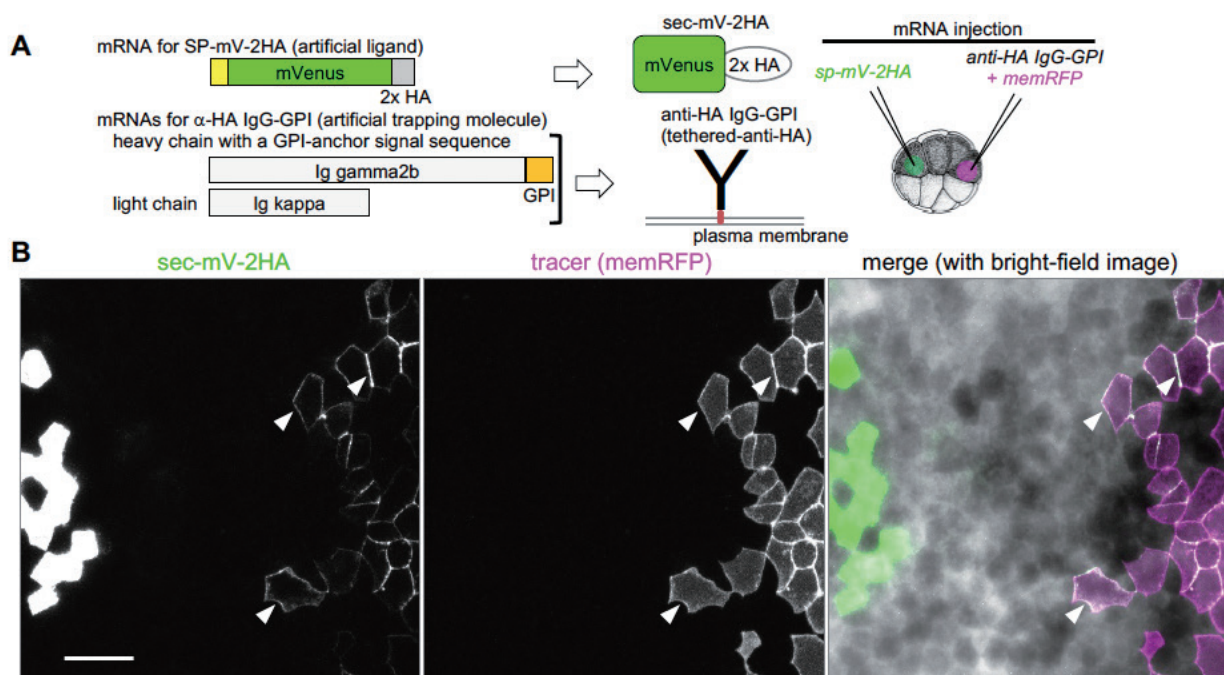


Figure 2. Visualization of dispersed proteins by an antibody-based ligand trapping: A. Schematic representation of tethered-anti-HA Ab, which can trap diffusing proteins with Venus tag. B. Results of trapping. The artificial ligand (sec-mV-2HA) was trapped at tethered-anti-HA Ab-expressing cells, distant from the source. The superficial layer of a *Xenopus* gastrula (st. 11.5) was imaged as a z-stack and its maximum intensity projection (MIP) was presented for the fluorescent images. Intercellular mVenus signal (green) of sec-mV-2HA was not apparent in the vicinity of source cells, but was detected around the tethered-anti-HA Ab-expressing cells (arrowheads) that are traced with memRFP (magenta).

odically 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. After this, the spatial pattern of somites is defined based on the temporal periodicity created by the clock. We are currently investigating the transitory mechanism concerning the temporal periodicity in the spatial patterns of somites using zebrafish embryos.

In addition to somites, we examined the development of another metameric structure, the pharyngeal arches (PA), which give rise to skeletal elements of jaws and gills. This metamerism is brought about by the segmental development of the pharyngeal pouches (PP), which are generated by outpocketing of the pharyngeal endoderm. Interestingly, the two most anterior PAs are likely to have different characteristics from the posterior PAs. The anterior PAs express genes involved in myogenesis and chondrogenesis whereas the posterior PAs do not undergo myogenesis and chondrogenesis in chick and mouse embryos. Furthermore, several studies have suggested that distinct segmentation mechanisms for anterior and posterior PAs may cooperate in establishing the entire series of PAs. However, development of this complex PA segmentation has been poorly understood until now. It has been quite puzzling how the seamless array of pharyngeal arches is generated by a combination of anterior and posterior PAs, which are formed by distinct mechanisms.

To address this issue, we examined the development of zebrafish pharyngeal endoderm, focusing in particular on the formation of PP2, which is located at the boundary between the anterior and posterior PAs. Precise examination by live-imaging and cell-tracing experiments performed in zebrafish showed that PP2 was formed in an unexpected manner. Rostral and caudal aspects of PP2 were initially formed separately, then subsequently established contact through the dynamic remodeling of the endoderm epithelium, upon which they finally became integrated (Figure 3). These results provide an answer to the question of how a seamless array of PAs are generated.

## Publication List:

### [Original papers]

- Okada, K., and Takada, S. (2020). The second pharyngeal pouch is generated by dynamic remodeling of endodermal epithelium in zebrafish. *Development* 147. DOI: 10.1242/dev.194738
- 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
- Uda, Y., Miura, H., Goto, Y., Yamamoto, K., Mii, Y., Kondo, Y., Takada, S., and Aoki, K. (2020). Improvement of phycocyanobilin synthesis for genetically encoded phytochrome-based optogenetics. *ACS Chem. Biol.* 15, 2896–2906. DOI: 10.1021/acscchembio.0c00477
- Yasuda H, Yamamoto H, Hanamura K, Mehruba M, Kawamata T, Morisaki H, Miyamoto M, Takada S, Shirao T, Ono Y, Mukai H. (2020) PKN1 promotes synapse maturation by inhibiting mGluR-dependent silencing through neuronal glutamate transporter activation. *Commun. Biol.* 3, 710. doi: 10.1038/s42003-020-01435-w.

### [Review articles]

- Mii, Y. (2020). Heparan Sulfate Clusters Regulate Distribution and Signaling of Wnt Morphogens. *Trends Glycosci. Glycotechnol.* 32, E205–E211. DOI: 10.4052/tigg.2006.7e
- Mii, Y., and Takada, S. (2020). Heparan sulfate proteoglycan clustering in Wnt signaling and dispersal. *Front. CELL Dev. Biol.* 8. DOI: 10.3389/fcell.2020.00631

## DIVISION OF EMBRYOLOGY



Professor  
FUJIMORI, Toshihiko

Assistant Professor: KOYAMA, Hiroshi  
NONOMURA, Keiko

Technical Staff: OKA, Sanae

Postdoctoral Fellow: KISHI, Kanae

SOKENDAI Graduate Student: KATAGIRI, Saki  
USAMI, Fumiko  
SAKURAI, Jun

Visiting Graduate Student: KATSUTA, Hiroki

Visiting Undergraduate: MIKOSHIBA, Seiya

Visiting Scientist: ARATA, Masaki

INOUE, Yuuki

Technical Assistant: HIGUCHI, Yoko

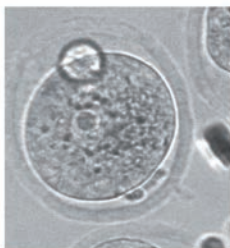
KANIE, Akemi

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. Combining these two types of reporter transgenic mouse lines, we have been analyzing the behaviors of cells and comparing gene expression properties at the single cell level. We found that cells expressing *Cdx2* exhibit plasticity of specification to TE and ICM lineages through positional changes.

## II. Histological observation of mouse embryos developing in the uterus

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



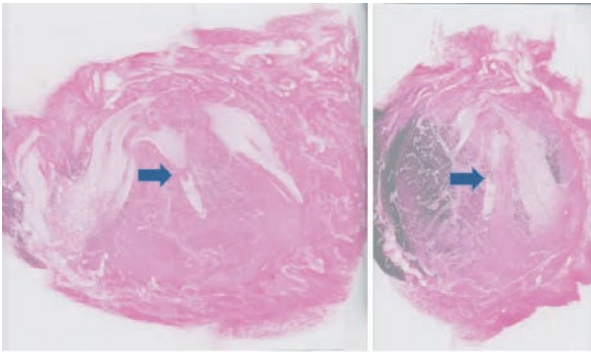


Figure 1. Three-dimensional image reconstruction of the pregnant uterus. A uterus obtained 5 days after fertilization was sectioned and images were digitized by an image scanner, and reconstructed three-dimensionally to observe the relationship between the embryo and the maternal tissue. The right panel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

### III. Polarity formation in the mouse oviduct epithelium

The oviduct (Fallopian tube) is the organ connecting the periovarian space and the uterine horn. The ova released from the ovary are transported through the oviduct, where the ova are fertilized by spermatozoon entering from the uterus. The epithelium of the mouse oviduct consists of multi-ciliated cells and secretory cells. Ovulated oocytes are transported in oviducts by unidirectional motility of multicilia. 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 evolutionarily 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 disrupt-

ed. 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.

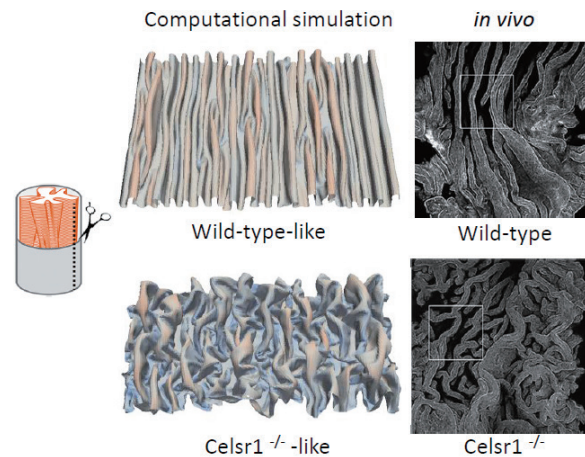


Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

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

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

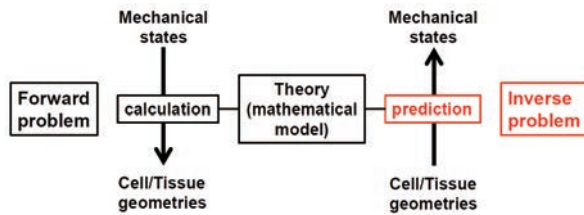


Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

## V. Mammalian tissue morphogenesis requiring mechanosensor channel PIEZOs

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

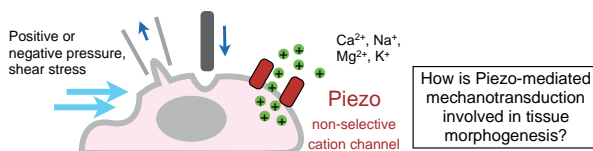


Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

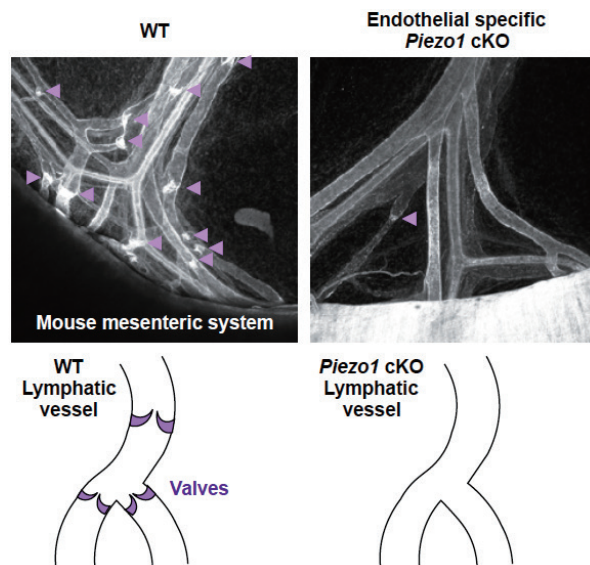


Figure 5. Reduced number of lymphatic valves in mice lacking PIEZO1 in endothelial cells.

mechanical forces, PIEZO activation, cellular responses, and tissue morphogenesis.

## VI. Mechanics of cell population patterning during development

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

### Publication List:

#### [Original papers]

- Hamashima, T., Ishii, Y., Nguyen, L.Q., Okuno, N., Sang, Y., Matsushima, T., Kurashige, Y., Takebayashi, H., Mori, H., Fujimori, T., *et al.* (2020). Oligodendrogenesis and myelin formation in the forebrain require platelet-derived growth factor receptor- $\alpha$ . *Neuroscience* 436, 11–26. DOI: 10.1016/j.neuroscience.2020.04.001
- Koyama, H., and Fujimori, T. (2020). Isotropic expansion of external environment induces tissue elongation and collective cell alignment. *J. Theor. Biol.* 496. DOI: 10.1016/j.jtbi.2020.110248
- Matsumoto, Y., Yamaguchi, Y., Hamachi, M., Nonomura, K., Muramatsu, Y., Yoshida, H., and Miura, M. (2020). Apoptosis is involved in maintaining the character of the midbrain and the diencephalon roof plate after neural tube closure. *Dev. Biol.* 468, 101–109. DOI: 10.1016/j.ydbio.2020.09.015
- Nguyen, Q.L., Okuno, N., Hamashima, T., Dang, S.T., Fujikawa, M., Ishii, Y., Enomoto, A., Maki, T., Nguyen, H.N., Nguyen, V.T., *et al.* (2021). Vascular PDGFR- $\alpha$  protects against BBB dysfunction after stroke in mice. *Angiogenesis* 24, 35–46. DOI: 10.1007/s10456-020-09742-w
- Sakai, Y., Kato, K., Koyama, H., Kuba, A., Takahashi, H., Fujimori, T., Hatta, M., Negri, A.P., Baird, A.H., and Ueno, N. (2020). A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, *Acropora tenuis*. *Sci. Rep.* 10. DOI: 10.1038/s41598-020-74649-x
- Usami, F.M., Arata, M., Shi, D., Oka, S., Higuchi, Y., Tissir, F., Takeichi, M., and Fujimori, T. (2021). Intercellular and intracellular cilia orientation is coordinated by CELSR1 and CAMSAP3 in oviduct multi-ciliated cells. *J. Cell Sci.* 134. DOI: 10.1242/jcs.257006

**DIVISION OF GERM CELL BIOLOGY**



Professor  
**YOSHIDA, Shosei**

Assistant Professor: **KITADATE, Yu**  
**NAKAGAWA, Toshinori**

Specially Appointed Assistant Professor:  
**HIRA, Seiji**

Technical Staff: **MIZUGUCHI, Hiroko**

JSPS Postdoctoral Fellow: **IKEDA, Tatsuro**

Research Staff: **HIRANO, Kodai**

**SATO, Toshiyuki**

SOKENDAI Graduate Student: **WANG, Zhe**

Visiting Undergraduate: **BABAGUCHI, Hirotaka**

Technical Assistant: **KON, Yayoi**

**MARUYAMA, Ayumi**

**FUJITA, Miyako**

**NISHIMURA, Chiaki**

Admin Support Staff: **KUBOKI, Yuko**



**Overview of our research**

The production of copious amounts of sperm over a long period in the testis is fundamental for the continuity of life across generations. The Division of Germ Cell Biology aims to understand the key processes of germ cell development, that lead to the robust production of sperm and the accurate transmission of genetic information.

In particular, our emphasis has been placed on the regulation and functional behavior of spermatogenic stem cells (SSCs) in the testes of mice. SSCs play a crucial role, not only as the origin of long-term sperm production, but also

as the crucible of genetic and potentially epigenetic mutations, the underpinning of evolution as well as congenital disease mechanisms. The principal and signature strategy of our research is to directly observe the behavior of individual germ cells *in vivo* in their natural (*i.e.*, physiological) state and take advantage of intravital live-imaging, lineage analysis, and biophysical analysis,

Our lab has figured out a number of key properties of mouse SSCs. One of these is that they include a functional hierarchy, comprised of an “actual” stem cell compartment that is able to self-renew, and a differentiation-primed, “potential” stem cell compartment. We have been investigating the cellular identity of “actual” SSCs, their *in vivo* behavior at a single-cell resolution, and their underlying mathematical principles, leading to the discovery of the dynamics of “population asymmetry” and subsequent “neutral competition” between SSCs. We have proposed that the aforementioned type of competition observed between SSCs could be the result of consumption of limited supply of self-renewal promoting extracellular ligands. The “potential stem cells” have also fascinated us. During steady-state spermatogenesis, these cells are largely active as transient amplifying cells and differentiate rather than self-renew. However, when tissues are insulted or testicular cells are transplanted into host testes, the probability of their self-renewal increases greatly. Such flexible and probabilistic features of stem cells have been found to be paradigmatic for many other stem cell-supported tissues. Our past key references related to these studies include Nakagawa *et al.*, *Dev. Cell* 2007; Yoshida *et al.*, *Science* 2007; Nakagawa *et al.*, *Science* 2010; Klein *et al.*, *Cell Stem Cell* 2010; Hara *et al.*, *Cell Stem Cell* 2014, Ikami *et al.*, *Development* 2015, Tokue *et al.*, *Stem Cell Reports* 2017, and Kitadate *et al.*, *Cell Stem Cell* 2019.

An important achievement we accomplished in 2020 was the revelation of SSC behavior in the context of post-transplantation reconstruction of spermatogenesis at a clonal resolution, and the reporting of a novel strategy to enhance the transplantation efficiency by tuning the post-transplantation fate of SSCs (Nakamura *et al.*, *Cell Stem Cell* 2021).

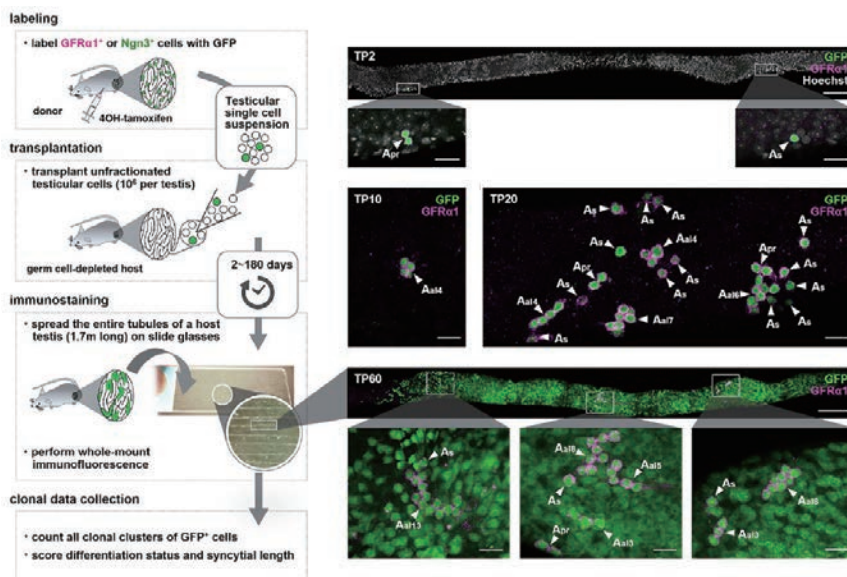


Figure 1. The “pulse-transplantation” experiment

In the donor mice, actual ( $GFR\alpha1+$ ) and potential ( $Ngn3+$ ) SSCs are pulse-labelled, whose testes were dissociated into single cell suspension for transplantation to germ cell-depleted hosts. After certain times, the entire testes of the hosts were recovered, untangled seminiferous tubules were processed for immunostaining. All the pulse-labelled ( $GFP+$ ) clones in the host testes were scored for statistical analyses. Adopted from *Cell Stem Cell* 5, 1443-1456 (2021).



## Post-transplantation regeneration: a remarkable feature of stem cells promising a wealth of applications

Tissue stem cells can restore the impaired structure and function of host tissues following transplantation. In blood, hematopoietic stem cell transplantation has been established as a radical treatment for leukemia, while trials to test the viability of stem cell transplantation in mesenchymal and epithelial tissues are ongoing. In male germline, SSC transplantation was established by Brinster and their colleagues in 1994 using mice, which promised a wealth of applications such as restoration of fertility to cancer patients after chemotherapy and the preservation of genetic diversity. However, the inefficiency of this technology currently rules out its practical application.

Indeed, our knowledge about the fate behavior of individual SSCs and their progenies after transplantation remains poorly understood, thus limiting the potential to develop new strategies to increase the currently low transplantation efficiencies.

## Clonal fate analysis of actual and potential stem cells following transplantation

A remarkable feature of SSCs that our group has discovered is the presence of “potential stem cells”, which significantly contributes to the post-transplantation regeneration in a manner similar to “actual stem cells” (Nakagawa *et al.*, 2007). Therefore, based on an experimental setting of pulse-labeling actual and potential SSCs using the specific expression of GFR $\alpha$ 1 and Ngn3 in these cells, respectively, we first investigated their detailed clonal fate following transplantation in the host testes (Figure 1). All the clones that had been derived from labelled SSCs and resided in the host testes were counted and scored for the number of constituent SSCs and differentiated cells over a broad time line spanning from 2 days to 180 days after transplantation, when single donor cell-derived clones formed robust repopulating colonies.

## Actual and potential SSCs follow indistinguishable clonal fates with comparable repopulation efficiencies

It was clear that single SSC-derived clones follow highly divergent fates, showing varying clone sizes (*e.g.*, the number of SSCs in individual clones, or physical extension within the tissue), rather than showing a stereotypical pattern. The clonal fate data derived from actual (GFR $\alpha$ 1<sup>+</sup>)

and potential (Ngn3<sup>+</sup>) SSCs first show that these cells form long-term repopulating colonies at comparable, albeit not equal, efficiencies. When Ngn3<sup>+</sup> cells were transplanted, a significant fraction of them were quickly converted into GFR $\alpha$ 1<sup>+</sup> cells within 2 days; an observation consistent with the results of the preceding studies. Interestingly, GFR $\alpha$ 1<sup>+</sup> cell- and Ngn3<sup>+</sup> cell-derived clones evolve parallel to indistinguishable size distributions and kinetics over time. Thus, actual and potential SSCs are equipotent in terms of clonal evolution during post-transplantation repopulation, irrespective of the original state on transplantation.

## Most of the initially settled SSCs are lost through stochastic differentiation and cell death

We subsequently looked in detail into the clonal fates of donor SSCs. We unexpectedly found that the number of SSCs that initially resided in the tissue was considerably larger than those which eventually formed colonies over the long term (*i.e.*, after couple of months). After being attached to the host tissue and moved to the location in which SSCs are normally found (on the basement membrane of seminiferous tubules), the vast majority of SSCs were lost through differentiation and cell death, with only a small fraction (one out of tens) contributing to long-term repopulation.

Furthermore, in collaboration with the Ben Simon Lab at the University of Cambridge, we asked whether this variable and intricate fate behavior can be explained by a minimal mathematical model, relying on an assumption that all SSCs are equivalent, and select their fates probabilistically at defined rates reliant only on their current state (*i.e.*, unrelated to the past history). Significantly, such simple models were found to be capable of predicting the broad range of clonal fate data with high accuracy, supporting the aforementioned model assumption that all SSCs are equipotent. This insight is in stark contrast with the prevailing thought that only a small fraction of GFR $\alpha$ 1<sup>+</sup> and/or Ngn3<sup>+</sup> cells are definitive SSCs that form colonies at high probabilities (presumptively, 100%).

## Post-transplantation fate of SSCs can be modified through differentiation inhibition

The suggestion by the modeling analysis that all SSCs are equivalent motivated us to test whether SSC fate can be altered to increase repopulation efficiency. In particular,

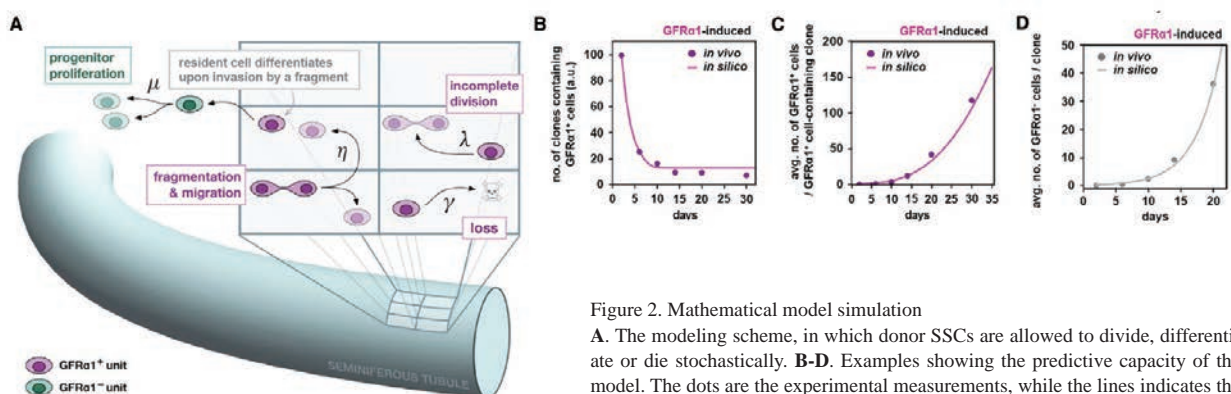


Figure 2. Mathematical model simulation

A. The modeling scheme, in which donor SSCs are allowed to divide, differentiate or die stochastically. B-D. Examples showing the predictive capacity of the model. The dots are the experimental measurements, while the lines indicates the simulation. Adopted from Cell Stem Cell 5, 1443-1456 (2021).

given that irreversible commitment of SSCs to differentiate and lose their self-renewing potential is induced by, and dependent on, retinoic acid (RA) signaling, we asked if the administration of a chemical inhibitor of retinoic acid (RA) synthesis, WIN18,446, can affect the behavior of transplanted SSCs. We found that WIN18,446 treatment can, in an *in vivo* context, not only inhibit differentiation of donor SSCs, but also incline them toward self-renewal and significantly increase the number of self-renewing pools of surviving SSCs. Such an effect was observed more dramatically in Ngn3<sup>+</sup> cells that were primed for differentiation, and effectively recruited to self-renewing pool by WIN18,446 treatment.

### Transient suppression of donor SSC differentiation boosts long-term repopulation and restores host fertility

Finally, we examined if the overall long-term repopulation efficiency could be increased, after this type of differentiation inhibition is terminated so as to allow the SSCs to proceed with spermatogenesis. Remarkably, we observed a dramatic increase in long-term repopulation efficiency, (*i.e.*, by 5-10 times) which was capable of restoring the fertility of a host that could not sire offspring through natural mating. Pups obtained from the donor SSC-derived sperm generated in the WIN18,446-treated host grew into healthy and fertile adults without any apparent abnormalities.

These results not only support the thesis that all SSCs are equipotent, but also offer a novel strategy to increase the transplantation efficiency through tuning the donor cell fate by changing the hosts' niche environment. While WIN18,446 treatment is effective, in theory, this efficiency could be further increased another ten times if all transplanted SSCs successfully repopulate. Technologically, the use of WIN18,446 and/or other reagents modifying the SSC fate shows promise in the field of SSC transplantation across broader applications, from restoring fertility to cancer patient after therapy to the preservation of genetic diversity.

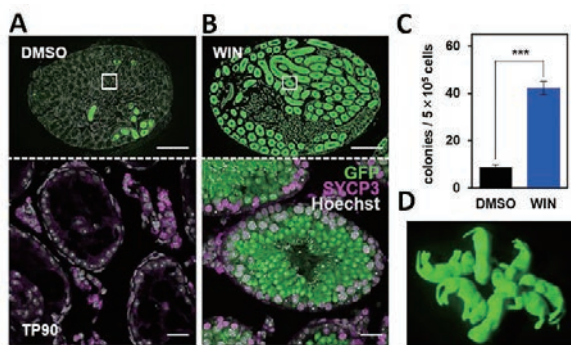


Figure 3. Restoration of host fertility using WIN18,446  
**A-B.** Representative appearance of the host testes 90 days after transplantation of GFP<sup>+</sup> donor testicular cells, in control (DMSO) and WIN18,446-treated hosts. Note the dramatic increase of the repopulation efficiency. **C.** Quantification of the repopulation efficiency based on the number of repopulating colonies. **D.** Offspring sired by the WIN18,446-treated host through natural mating with wild-type female. The green fluorescence indicates that the pups were originated from GFP<sup>+</sup> donor testicular cells developed into functional sperm in the host testes. Adopted from Cell Stem Cell 5, 1443-1456 (2021).

### Publication List:

#### [Original Papers]

- Rezende-Melo, C.A., Caldeira-Brant, A.L., Drumond-Bock A. L. and Buchold, G.M., Shetty, G., Almeida, F.R.C.L., Matzuk, M.M., Hara, K., Yoshida, S., Meistrich, M.L., and Chiarini-Garcia, H. (2020). Spermatogonial asynchrony in Tex14 mutant mice lacking intercellular bridges. *Reproduction* 160, 205–215. DOI: 10.1530/REP-20-0118
- Sznurkowska, M.K., Hannezo, E., Azzarelli R., Chatzeli, L., Ikeda, T., Yoshida, S., Philpott, A., and Simons, B.D. (2020). Tracing the cellular basis of islet specification in mouse pancreas. *Nat. Commun. 11*. DOI: 10.1038/s41467-020-18837-3
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#### [Review Article]

- Yoshida, S. (2020). Mouse spermatogenesis reflects the unity and diversity of tissue stem cell niche systems. *Cold Spring Harb. Perspect. Med. 10*. DOI: 10.1101/cshperspect.a036186

## 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 said abilities from non-regenerative animals. We have already succeeded in achieving this with planarians, which were able to regenerate their heads through RNAi (Umesono *et al.*, 2013 Nature), and accomplishing functional joint regeneration in frogs through the activation of reintegration systems (Tsutsumi *et al.*, 2016 Regeneration).

We are currently trying to induce limb regeneration abilities in frogs, as they lose the capability to achieve complete limb regeneration after metamorphosis. We are now focusing on the *Sonic hedgehog* (*Shh*) super-enhancer MFCS1 (mammals-fishes conserved sequence 1), since it has been suggested that the loss of MFCS1 activity after metamorphosis might cause a failure in achieving the aforementioned limb regeneration in adult frogs (Yakushiji *et al.*, 2009). When we compared the MFCS1 sequences between newts and frogs, newts were found to possess several specific sequences (Figure 1), suggesting that sequence differences might affect super-enhancer formation between newts and frogs after metamorphosis.

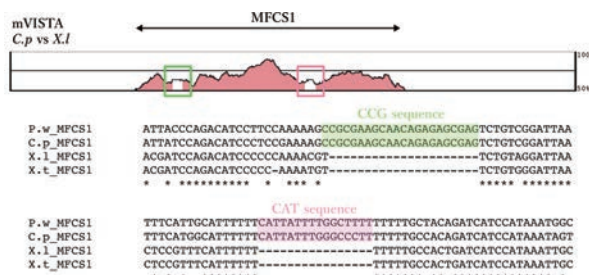


Figure.1 Comparison of the MFCS1 sequences between newts and frogs.

We subsequently tried to detect enhancer RNA (eRNA) which might be transcribed in the MFCS1 region. An interesting aspect of this was that eRNA was detected in regenerating blastema of adult newt from st. 2.0 (*Pleurodeles waltl*: Figure 2A).

Conversely, expression of eRNA was suppressed in the frog's blastema (*Xenopus laevis*) after metamorphosis (Figure 2B). Thus, we subsequently planned to swap the MFCS1 sequences between newts and frogs using CRISPR/Cas9 technology, expecting that the eRNA will be transcribed from the newt's MFCS1 after swapping in frogs.

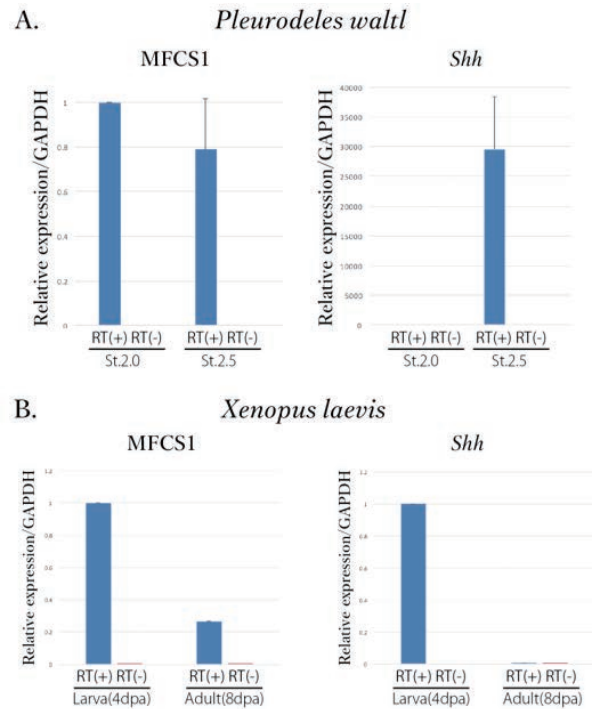


Figure.2 Expression of MFCS1-eRNA and *Shh* mRNA in the regenerating blastema of newt (*P.w.*) and frog (*X.l.*)

(A) The left and right panels show expression levels of MFCS1-eRNA and *Shh* mRNA, respectively. The left and right graphs in each panel were obtained from regenerating blastema at St.2.0 and St.2.5 of adult newts after amputation.

(B) The left and right panels shows expression levels of MFCS1-eRNA and *Shh* mRNA in frogs before and after metamorphosis.

### Trials in isolating viable adult pluripotent stem cells derived from planarian using FACS

We have tried to develop an isolation method for viable adult pluripotent stem cells (aPSC) from planarians using FACS and succeeded in conditioning the low toxic staining method with both nuclear and cytoplasmic fluorescence dyes. 1µM Hoechst 33342 or 0.05 µg/ml Calsein AM could be used for isolating viable aPSC of the planarian, *Dugesia japonica*. We are presently investigating the effects of FGF- and Wnt-morphogens to cultured aPSC to demonstrate “the double gradient hypothesis”, which was proposed by Thomas Hunt Morgan (Morgan, 1901) and our group (Umesono *et al.*, Nature, 2013).



## Publication List:

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### [Original papers]

- Auwal, M.A., Kashima, M., Nishimura, O., Hosoda, K., Motoishi, M., Kamimura, A., Okumura, A., Agata, K., and Umesono, Y. (2020). Identification and characterization of a fibroblast growth factor gene in the planarian *Dugesia japonica*. *Dev. Growth & Differ.* 62, 527–539. DOI: 10.1111/dgd.12696
- Hijioka, M., Ikemoto, Y., Fukao, K., Inoue, T., Kobayakawa, T., Nishimura, K., Takata, K., Agata, K., and Kitamura, Y. MEK/ERK signaling regulates reconstitution of the dopaminergic nerve circuit in the planarian *Dugesia japonica*. *Neurochem. Res.* DOI: 10.1007/s11064-020-03226-5
- Miura, S., Tsutsumi, R., Agata, K., and Endo, T. (2020). Maturing articular cartilage can induce ectopic joint-like structures in neonatal mice. *Regen. Eng. Transl. Med.* 6, 373–382. DOI: 10.1007/s40883-020-00176-w
- Sonpho, E., Wootthichairangsan, C., Ishida, M., Inoue, T., Agata, K., Maleehuan, A., Charngkaew, K., Chomane, N., Moonsom, S., Wongtrakongate, P., *et al.* (2020). ECM-body: A cell-free 3D biomimetic scaffold derived from intact planarian body. *Zoolog. Sci.* 37, 307–313. DOI: 10.2108/zs190135

### [Review article]

- Kashima, M., Agata, K., and Shibata, N. (2020). What is the role of PIWI family proteins in adult pluripotent stem cells? Insights from asexually reproducing animals, planarians. *Dev. Growth & Differ.* 62, 407–422. DOI: 10.1111/dgd.12688

**DIVISION OF BEHAVIORAL NEUROBIOLOGY**



Professor  
**HIGASHIJIMA, Shin-ichi**

Assistant Professor: **KIMURA, Yukiko**  
**TANIMOTO, Masashi**  
 Technical Staff: **TAKEUCHI, Yasushi**  
 JSPS Postdoctoral Fellow: **SUZUKI, Daichi**  
 SOKENDAI Graduate Student: **SUGIOKA, Takumi**  
**KAWANO, Kohei**  
**SHIMIZU, Sayaka**  
 Visiting Graduate Student: **UEMURA, Yuto**  
 Visiting Undergraduate: **KATAYAMA, Taisei**  
 Technical Assistant: **TERASAWA, Yoko**  
**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).

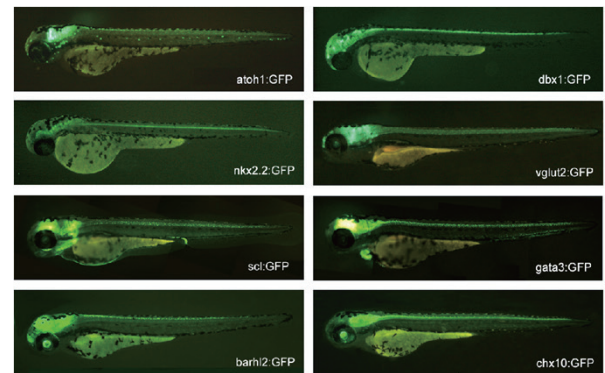


Figure 1. Examples of transgenic fish expressing GFP in specific classes of neurons.

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. Neuronal circuits that control rhythmic pectoral fin movements**

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

circuits that control rhythmic pectoral fins in more detail.

We performed electrophysiological recordings of Ab motoneurons (MNs) and Ad MNs during fictive swimming. Both Ab MNs and Ad MNs show rhythmic spiking activities (Figure 4). Activities of Ab MNs and Ad MNs on the same side alternated. Voltage clamp recordings showed that both Ab MNs and Ad MNs received alternating excitatory and inhibitory inputs during swimming cycles. Excitation mainly occurred in their preferential firing phase, and inhibition mainly occurred for the rest of the period. To obtain insights into the source of these inputs, we are now investigating the timing of spiking activities in possible premotor interneurons.



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

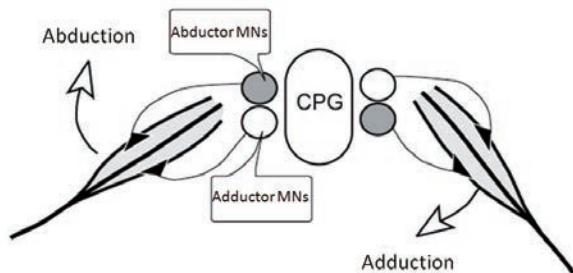


Figure 3. Schematic of rhythmic movements of pectoral fins during swimming. CPG, Central Pattern Generator.

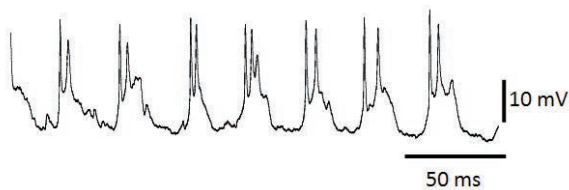


Figure 4. Rhythmic firings of an abductor motoneuron during swimming.

### III. *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 5). 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 direc-

tion- 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.

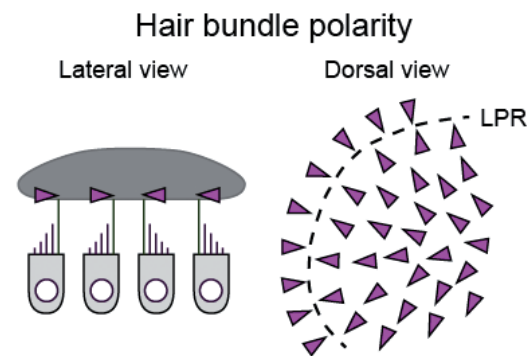


Figure 5. Hair bundle polarity (arrowheads) in the utricle.

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 6). 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, jRCaMP7f, and red fluorescent protein, tdTomato, in the hair cells.

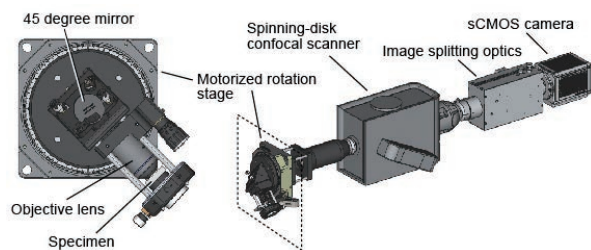


Figure 6. Tiltable objective microscope.

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 7). 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.



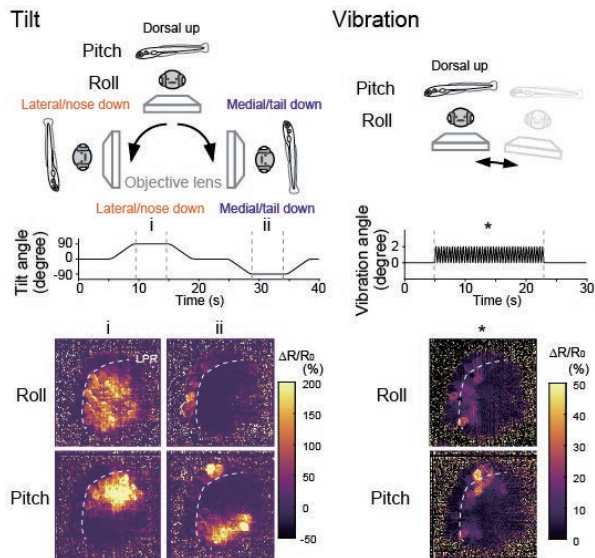


Figure 7. Hair cell responses to head tilt or vibration in the utricle

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.

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

Coordinated movements of the left and right side of the body is critical for any types of locomotion including walking, flying and swimming. The coordination is mainly mediated by commissural neurons located in the spinal cord. In fish swimming, it is known that commissural inhibitory neurons in the spinal cord ensure left-right alternating movements. The developmental origin of these commissural inhibitory neurons, however, has been elusive. We investigated anatomy and function of two commissural inhibitory neuron types, *dI6dmrt3a* and *V0d*, derived from the *pd6* and *p0* progenitor domains, respectively. We found that both of these commissural neuron types have monosynaptic, inhibitory connections to neuronal populations active during swimming, supported their role in providing inhibition to the contralateral side. *V0d* neurons tend to fire during faster and stronger movements, while *dI6dmrt3a* neurons tend to fire more consistently during swimming. Ablation of *dI6dmrt3a* neurons leads to an impairment of left-right alternating activity through abnormal co-activation of motor neurons on both sides of the spinal cord. Our results suggest that *dI6dmrt3a* and *V0d* commissural inhibitory neurons synergistically provide inhibition to the opposite side across different swimming behaviors (Figure 8).

#### Commissural inhibition during swimming

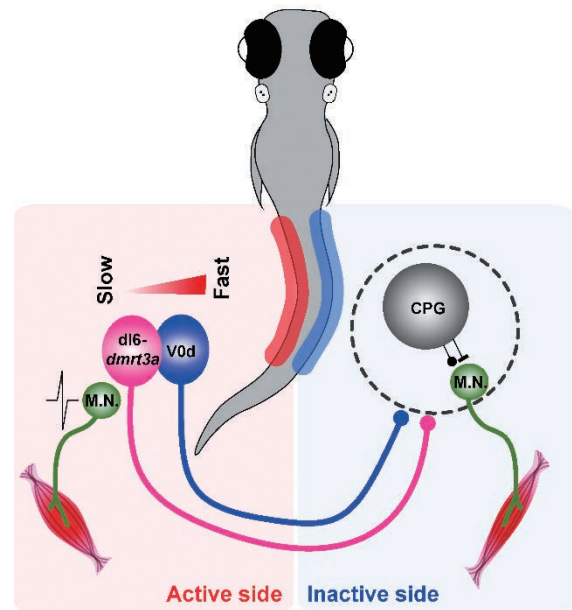


Figure 8. Summary of the proposed role of *dI6dmrt3a* and *V0d* neurons during swimming. CPG, central pattern generator

#### Publication List:

##### [Original Papers]

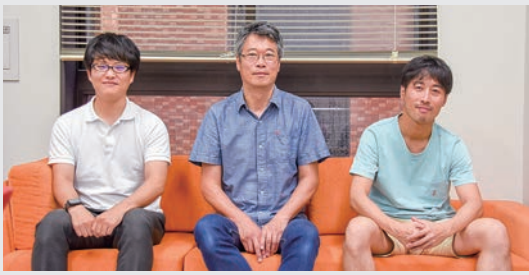
- Liu, Z., Kimura, Y., Higashijima, S., Hildebrand, D.G.C., Morgan, J.L., and Bagnall, M.W. (2020). Central vestibular tuning arises from patterned convergence of otolith afferents. *Neuron* 108, 748+. DOI: 10.1016/j.neuron.2020.08.019
- Mizoguchi, T., Fukada, M., Iihama, M., Song X., Fukagawa, S., Kuwabara, S., Omaru, S., Higashijima, S., and Itoh, M. (2020). Transient activation of the Notch-her15.1 axis plays an important role in the maturation of V2b interneurons. *Development* 147. DOI: 10.1242/dev.191312
- Uemura, Y., Kato, K., Kawakami, K., Kimura Y., Oda, Y., and Higashijima, S. (2020). Neuronal circuits that control rhythmic pectoral fin movements in zebrafish. *J. Neurosci.* 40, 6678–6690. DOI: 10.1523/JNEUROSCI.1484-20.2020

**LABORATORY OF NEUROPHYSIOLOGY**

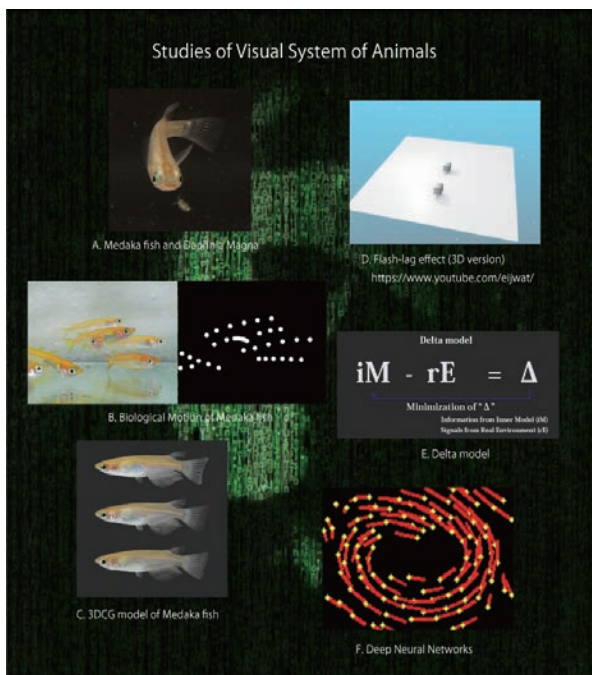


Associate Professor  
**WATANABE, Eiji**

*Specially Appointed Assistant Professor :*  
**KOBAYASHI, Taisuke**  
*JSPS Postdoctoral Fellow: NISHIUMI, Nozomi*  
*Technical Assistant: WATABE, Mihoko*  
**TSURUMARU, Mayumi**



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



Visual overview of this lab's work.

**I. Psychophysical study of medaka fish**

One of our major subjects is the psychophysical and computational study of medaka (*Oryzias latipes*, Matsunaga & Watanabe, 2010). We have recently made progress in studies of prey-predator interaction using both these organisms and zooplankton. Visual motion cues are one of the most important factors for eliciting animal behaviors, includ-

ing predator-prey interactions in aquatic environments. To understand the elements of motion that cause such selective predation behavior, we used a virtual plankton system where we analyzed the predation behavior in response to computer-generated plankton. To achieve this, virtual plankton models were programmed on a computer and presented to predator medaka. As a result of this analysis, we confirmed that medaka exhibited predation behavior against several characteristic movements of the virtual plankton, particularly against a swimming pattern that could be characterized as a pink noise motion. The analysis of predator-prey interactions via pink noise motions will be a research field of great interest moving forward (Matsunaga & Watanabe, 2012).

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

Additionally, we have developed a novel method for behavior analysis using 3D computer graphics (Nakayasu *et al.*, 2017). The fine control of various features of living fish has been difficult to achieve in studies of their behavior. However, computer graphics allow us to systematically manipulate morphological and motion cues. Therefore, we have constructed 3D computer graphic animations based on tracking coordinate data and photo data obtained from actual medaka (Figure 1). These virtual 3D models will allow us to represent medaka more faithfully as well as undertake a more detailed analysis of the properties of the visual stimuli that are critical for the induction of various behaviors. This experimental system was applied to studies on dynamic

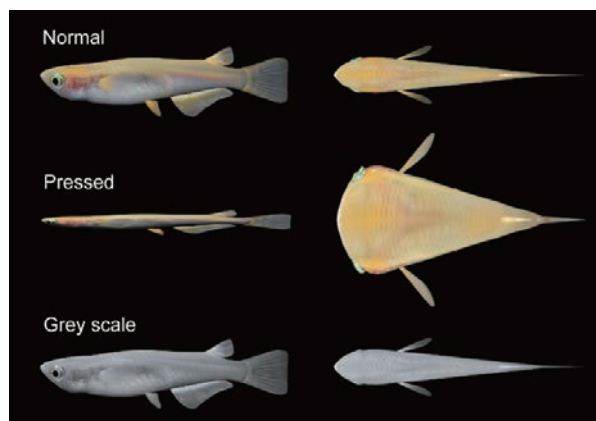


Figure 1. Virtual medaka fish.



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).

## II. Psychophysical study of human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual illusions experienced by human beings. One recent focus of this debate is the flash-lag effect, in which a moving object is perceived to head towards a flashed object when both objects are aligned in an actual physical space. We developed a simple conceptual model explaining the flash-lag effect (Watanabe *et al.*, 2010). In recent years, we have made more developed novel visual illusions, such as the shelf-shadow illusion (3rd Award of the 5th Illusion Contest, <https://doi.org/10.6084/m9.figshare.6137558>), the Monstre Benham illusion (2nd Award of the 11th Visual Illusion and Auditory Illusion

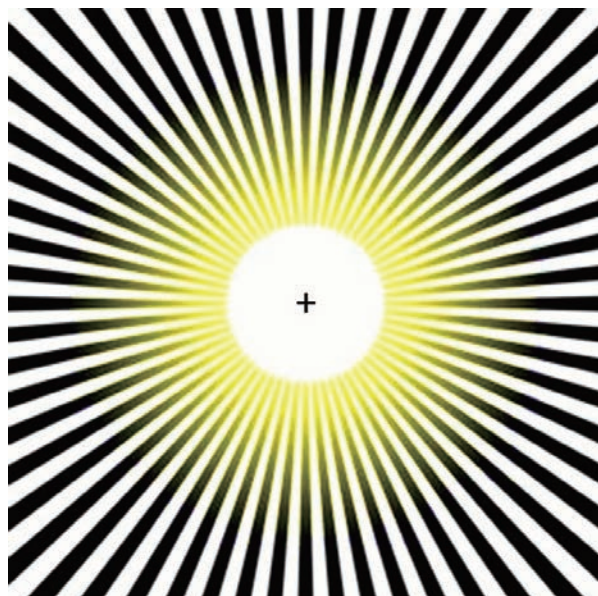


Figure 2. Morning Monster Illusion. Watanabe (National Institute for Basic Biology) and Sinapayen (Sony CSL) got 10th place at the 12th Visual Illusion and Auditory Illusion Contest, for the Morning Monster Illusion. It is one of the color aftereffect induced by glare effect. (demo, <https://ewatanabe.blogspot.com/2020/11/monstre-matin-illusion-10th-award-of.html>).

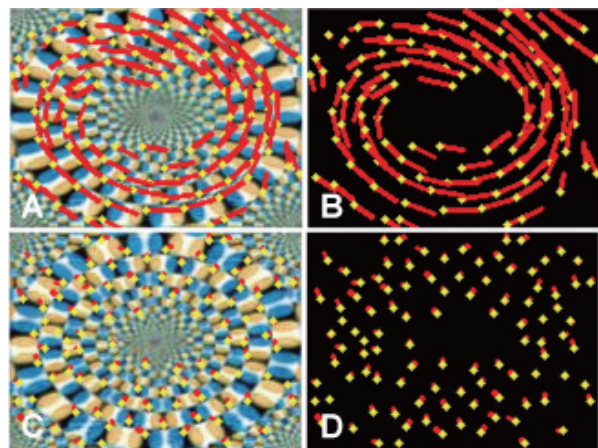


Figure 3. Optical flow vectors detected in the illusion. Red bars denote the direction and magnitude of vectors, yellow dots denote the start points of the vectors. A and B: Illusion, C and D: Non-Illusion.

Contest, <https://doi.org/10.6084/m9.figshare.10046534>), and the Morning Monster Illusion (<https://doi.org/10.6084/m9.figshare.13125518>, Figure 2).

In 2018, we successfully generated deep neural networks (DNNs) that represent the perceived rotational motion for illusion images that were not physically moving, yet similar to what we experience in human visual perception. (Figure 3, Watanabe *et al.*, 2018). These DNN computer models will help to facilitate our future work on perception science. This experimental system was applied to studies on evolutionary illusion generator in collaborating with Dr. Lana Sinapayen (Sony CSL). (EIGen, [https://github.com/LanaSina/evolutionary\\_illusion\\_generator](https://github.com/LanaSina/evolutionary_illusion_generator). Sinapayen and Watanabe, 2020). Currently, a variety of research is being conducted using these AI systems (<https://arxiv.org/abs/2106.09979>, <https://arxiv.org/abs/2106.12254>).

## III. Ecological study of tactics in predators and prey

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

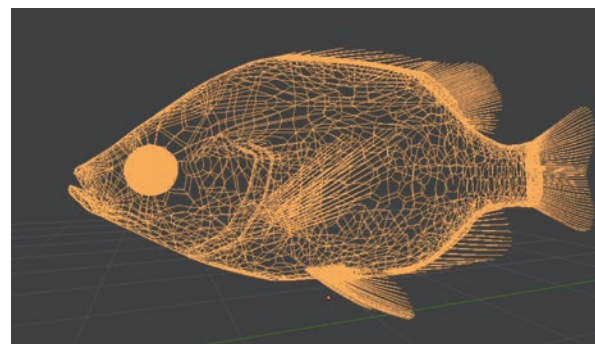


Figure 4. A computer animation of the virtual predatory fish.



## 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: AOYAMA, Tsuyoshi  
NARUKAWA, Hideki  
PALFALVI, Gergo

SOKENDAI Graduate Student: SUDA, Hiraku  
HORIUCHI, Yuta  
DE VILLIERS, Ruan  
CHEN, Peng  
UEDA, Masamichi

Visiting Graduate Student: TANASE, Kuniaki

Visiting Scientist: GU, Nan

Technical Assistant: AOKI, Etsuko  
FUKADA, Hatsumi  
HIRAMATSU, Mika  
KAJIKAWA, Ikumi  
MATSUZAKI, Yoko  
MASUOKA, Tomoko  
NISHI, Tayo  
ZHANG, Liechi  
OGAWA, Yuko  
KOJIMA, Yoko  
HASEBE, Yuki

Admin Support Staff:



## Reprogramming by STEMIN and DNA damage



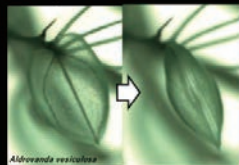
*Physcomitrium patens*



Pitcher leaf evolution

*Drosera muscipula*

## Action potentials and movement



*Aibowanda vesiculosa*



*Mimosa pudica*



*Drosera rotundifolia*

## I. Evolution of Complex Adaptive Characters

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

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

The cell division axis must be properly regulated during the development of both metazoans and land plants. Genetic changes in the regulation of the cell division axis lead to the development of multicellular organisms. Since they do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants most likely have different regulatory mechanisms. We aim to investigate the connecting factors between microtubules and GRAS transcription factors that regulate periclinal cell divisions in the moss *Physcomitrella patens*.

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

Regulation of local cell growth underlies the geometric shape formation of individual cells. Cell shape is an instructive factor in oriented cell division, which guides morphogenesis in land plants. Mr. Liechi Zhang, a graduate student in our lab, found that a transporter mutant, which belongs to the ABC gene family, exhibited a cell shape abnormality. We are presently investigating its underlying mechanism.

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

## III. DNA damage triggers reprogramming of differentiated cells into stem cells with STEMIN

Plants are better able to undergo cellular reprogramming from differentiated cells to stem cells compared to animals. The genetic regulatory networks of reprogramming induced by phytohormones and wounding accompanying cell death have been extensively studied. We previously investigated the molecular mechanisms underlying reprogramming

caused by wounding in the moss *Physcomitrella patens*. This year, we reported the unexpected identification of a new genetic regulatory pathway of reprogramming induced by moderate, repairable DNA damage in the absence of exogenous phytohormone treatment and wounding. When intact leafy shoots were transiently exposed to DNA damage-inducing reagents, the DNA damage was successfully repaired and the leaf cells changed to stem cells, which subsequently produced leafy shoots. Furthermore, we found that this pathway depends on the AP2/ERF transcription factor *STEMIN*, whose ectopic expression changes intact differentiated leaf cells into stem cells (Ishikawa *et al.* 2019 Nat. Plants 5: 681). Consequently, this response could be quite helpful to plants in nature. Since plants cannot immediately escape from severe environments that induce DNA damage, the conversion of differentiated cells into stem cells, which proliferate and produce new shoots, allows plants to escape from harsh environments to grow in safer places. Our findings shed light on an unforeseen pathway from the induction of DNA breaks to cellular reprogramming. An international joint graduate student Ms. Nan Gu from Dr. Chunli Chen's laboratory in the Huazhong Agricultural University mainly worked on this project (Gu *et al.* 2000. Nat. Plants).

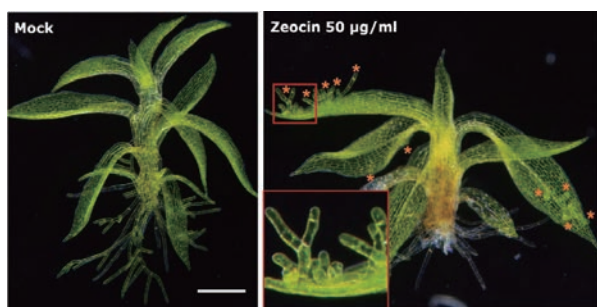


Figure 1. The DNA damage reagent zeocin induces reprogramming of leaf cells into stem cells.

#### IV. Evolution of Carnivory in Flowering Plants

Carnivorous plants attract, capture, digest, absorb, and nourish small animals with specialized leaves, so they can grow in nutrient poor environments where they can gain prominence over other plants. To better understand the evolution of botanical carnivory, we and a group of international collaborators sequenced draft genomes of the Venus flytrap, *Dionaea muscipula*, the waterwheel plant, *Aldrovanda vesiculosa*, and the sundew *Drosera spatulata*. Mr. Gergo Plafalvi, a graduate student and colleagues compared the genomes and identified an early whole genome duplication in the family as a source for carnivory-associated genes. In particular, the recruitment of genes to the trap from the root was a major mechanism in the evolution of carnivory, supported by family-specific duplications. These genomes belong to the gene poorest land plants sequenced thus far, suggesting a reduction of selective pressure on different processes, including non-carnivorous nutrient acquisition (Plafavi *et al.* 2020 Curr. Biol.).

To investigate the molecular bases of carnivory, Dr. Hideki Narukawa performed mostly comparative analysis of carnivorous pitcher leaves and non-carnivorous flat leaves in

the carnivorous plant *Cephalotus follicularis*. We found that hollow formation, which was the first step in pitcher leaf development, was initiated by growth inhibition on the adaxial side of leaf primordia. This process may be regulated by the phytohormone cytokinin.

#### V. Calcium ions mediate memory in the Venus flytrap

The leaves of the carnivorous plant Venus flytrap, *Dionaea muscipula* (*Dionaea*) close rapidly to capture insect prey. The closure response usually requires two successive mechanical stimuli applied to the sensory hairs on the leaf blade within a span of approximately 30 seconds. An unknown biological system in *Dionaea* is thought to memorize the first stimulus and transduce the signal from the sensory hair to the leaf blade. A graduate student Hiraku Suda and his colleagues linked signal memory to calcium dynamics using transgenic *Dionaea* expressing a  $\text{Ca}^{2+}$  sensor. Stimulation of the sensory hair caused an increase in cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) starting in the sensory hair and spreading to the leaf blade. A second stimulus increased  $[\text{Ca}^{2+}]_{\text{cyt}}$  to an even higher level, meeting a threshold that is correlated to the leaf blade closure. Because  $[\text{Ca}^{2+}]_{\text{cyt}}$  gradually decreased after the first stimulus, the  $[\text{Ca}^{2+}]_{\text{cyt}}$  increase induced by the second stimulus was insufficient to meet the putative threshold for movement after about 30 seconds. The  $\text{Ca}^{2+}$  wave triggered by mechanical stimulation moved an order of magnitude faster than that induced by wounding in petioles of *Arabidopsis thaliana* and *Dionaea*. The capacity for rapid movement has evolved repeatedly in flowering plants. This study opens a path to investigate the role of  $\text{Ca}^{2+}$  in plant movement mechanisms and their evolution.

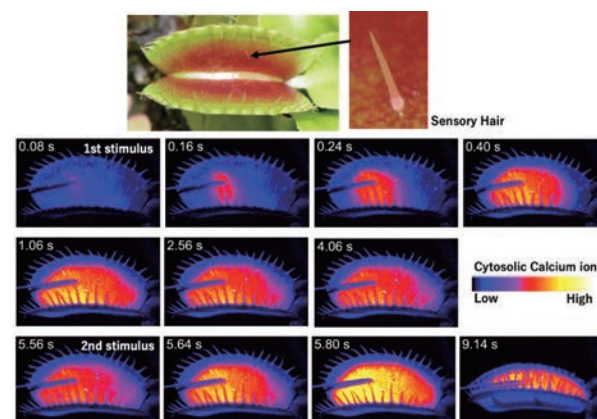


Figure 2. Fluorescence images of a GCaMP6f *Dionaea* leaf blade after a sensory hair was stimulated with a needle.

#### VI. Molecular mechanisms and evolution of the generation and transmission of action potential 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*, the wheel plant *Aldrovanda vesiculosa*, and the sundew *Drosera rotundifolia*. We use these four species, as well as *Arabidopsis thaliana*, to study the molecular mechanisms of action potential generation and transmission. We have obtained genome sequences for these species and established techniques for transformation. 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.

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#### [Original papers]

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- Yano, H., Alam, M.Z., Rimbara, E., Shibata, T.F., Fukuyo, M., Furuta, Y., Nishiyama, T., Shigenobu, S., Hasebe, M., Toyoda Atsushi and Suzuki, Y., *et al.* (2020). Networking and specificity-changing DNA methyltransferases in *Helicobacter pylori*. *Front. Microbiol.* *11*, 1628. DOI: 10.3389/fmicb.2020.01628

#### [Review article]

- Mano, H., and Hasebe, M. (2021). Rapid movements in plants. *J. Plant Res.* *134*, 3–17. DOI: 10.1007/s10265-020-01243-7



## DIVISION OF SYMBIOTIC SYSTEMS



Professor  
KAWAGUCHI, Masayoshi



Associate Professor  
SOYANO, Takashi

Assistant Professor: KAWADE, Kensuke

Specially Appointed Assistant Professor:

LIU, Meng

Technical Staff: FUKADA-TANAKA, Sachiko

Postdoctoral Fellow: TSUGANE, Mika

YANO, Koji

HASHIMOTO, Kayo

JSPS Postdoctoral Fellow: FUKUDOME, Mitsutaka

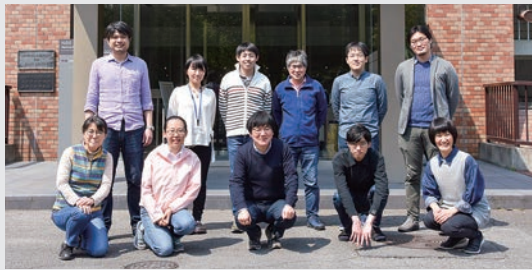
Visiting Scientist: NAKAGAWA, Tomomi

SOKENDAI Graduate Student: OKUMA, Nao

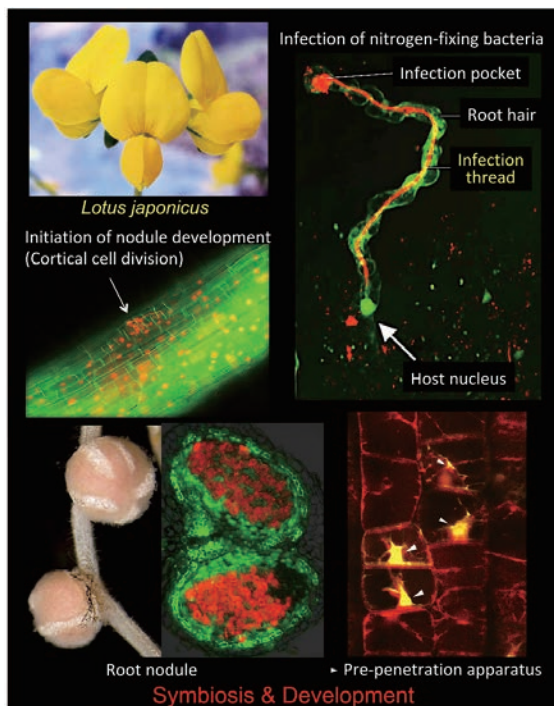
GOTO, Takashi

Technical Assistant:

ODA, Akiko



*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.



Visual overview of this lab's work.

However, more than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. Despite marked differences between the fungal and bacterial symbioses, common genes are required for both interactions. Using a model legume, *Lotus japonicus*, we are trying to elucidate the molecular mechanisms of both symbiotic systems.

## I. Root nodule symbiosis

### Systemic optimization of root nodulation through shoot-derived miR2111.

The symbiosis with rhizobia enables legumes to thrive under nitrogen-limiting conditions. However, since  $N_2$ -fixation is a highly energy-consuming process, excess nodule formation is detrimental to the host growth. Legumes, therefore, tightly control nodule numbers using a root-shoot-root, long-distance signaling mechanism: autoregulation of nodulation (AON) (Figure 1). In *Lotus japonicus*, the initial step of AON is the synthesis of root-derived mobile signals, CLV3/ESR-related (CLE) ROOT SIGNAL 1, 2, and 3 (CLE-RS1, 2, and 3) peptides, in response to either rhizobial infection or high nitrate concentrations in the roots. These CLE peptides are translocated into the shoot through xylem vessels and are perceived by a shoot-acting HYPERNODULATION ABERRANT ROOT1 (HAR1) receptor-like kinase. Consequently, TOO MUCH LOVE (TML) F-box/kelch repeat protein, expressing in roots, inhibits nodulation downstream of HAR1.

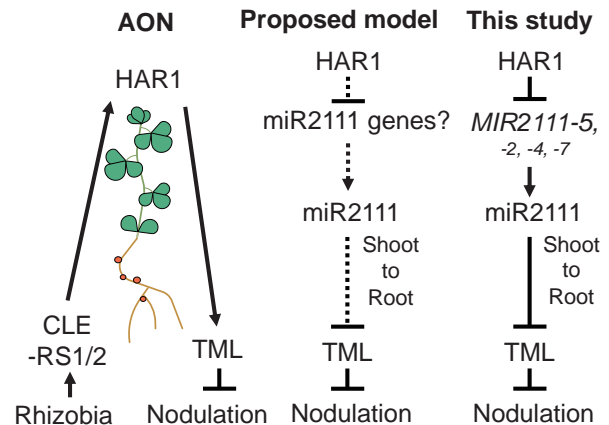


Figure 1. The model of AON and overview of this study. AON model (Left), proposed model of miR2111 function in AON by previous study (Middle), and demonstrated in our study.

In this signaling system, the detailed regulatory mechanism of nodulation by the shoot in AON remains unclear. A microRNA, miR2111, that targets *TML* mRNA, is the most plausible shoot-derived factor in AON (Figure 1). Since the promoter activity of one miR2111 gene, *MIR2111-3*, is detected predominantly in leaves, the shoot-to-root translocation of miR2111 has been postulated to explain the shoot-mediated control of nodulation. However, whether *MIR2111-3* is a responsible locus for AON remains unclear. Moreover, the role of shoot-accumulating miR2111s in the systemic regulation of nodulation is unproven thus far. Therefore, we investigated highly contributing miR2111 genes to AON and the function of shoot-accumulating miR2111 to clarify the regulatory mechanism of nodulation from the shoot.

Three miR2111 loci (*MIR2111-1* to *MIR2111-3*) have been reported in *L. japonicus*. To identify the loci responsible for AON, we first searched additional potential miR2111 genes and found four new miR2111 loci, *MIR2111-4* to *MIR2111-7*, on the *L. japonicus* genome through hairpin structure prediction by combining BLAST search and RNA-seq-based gene prediction. Of the seven miR2111 genes, *MIR2111-2*, *MIR2111-4*, *MIR2111-5*, and *MIR2111-7* were expressed in leaves, and the accumulation levels of these transcripts decreased after rhizobial inoculation in a HAR1-dependent manner (Figure 1). *MIR2111-2* and *MIR2111-5* overexpression in hairy roots suppressed *TML* mRNA accumulation and significantly increased nodule numbers, whereas that of *MIR2111-4* did not influence nodulation.

Of the seven miR2111 loci, *MIR2111-5* showed the highest levels of its primary transcripts in leaves. Thus, we hypothesized that *MIR2111-5* significantly contributes to the accumulation of mature miR2111s in leaves and roots. Using *MIR2111-5* promoter GUS assays, we found that *MIR2111-5* was expressed predominantly in the phloem of leaves. *mir2111-5* mutants reduced mature miR2111 levels in both leaves and roots to < 50% of those observed in the wild-type, and significantly decreased the nodule and infection thread numbers compared to those in the wild-type. Furthermore, grafting experiments demonstrated that wild-type rootstock grafted with *MIR2111-5*-overexpressing scion showed increased nodules and mature miR2111s levels (Figure 2). The production of mature miR2111s in leaves by *MIR2111-5* is therefore necessary for the systemic control of nodulation and mature miR2111 levels in roots. Taken together, we clearly showed the systemic effect of shoot-accumulating miR2111 on nodulation and determined that *MIR2111-5* is a highly contributing locus for AON.

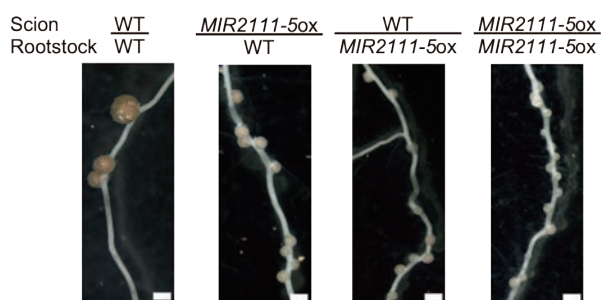


Figure 2. Reciprocal grafting between wild-type and *MIR2111-5ox* overexpression plants. Nodulation at 28 days after inoculation (dai) in wild-type and *MIR2111-5ox* rootstocks grafted with wild-type and *MIR2111-5ox* scions. Scale bars: 1 mm.

## II. Arbuscular mycorrhizal symbiosis

The roots of most terrestrial plants in the world have a symbiotic relationship with filamentous fungi via mycorrhizae. Approximately 80% of land plants including 94% of Angiosperms form some type of association with mycorrhizae. Arbuscular mycorrhizae, a type of endomycorrhiza in which fungal hyphae enters the plant cells and shapes highly branched structures named arbuscules, formed symbiotic relationships with land plants more than 400 million years ago. In contrast, land plant associations with

ectomycorrhizae, which is characterized by dense root-surrounding hyphae and intercellular hyphae between root cells, began about 190 million years ago. Even in modern ecosystems, arbuscular mycorrhizae constitute the most abundant form of mycorrhizal association with angiosperms. Thus, arbuscular mycorrhizal (AM) symbiosis is considered the most basic form of mycorrhizal symbiosis. AM symbiosis is evolutionarily and agriculturally important because land plants including bryophytes, pterophytes and many crops colonize with AM fungi to obtain nutrients from the soil (Figure 3).

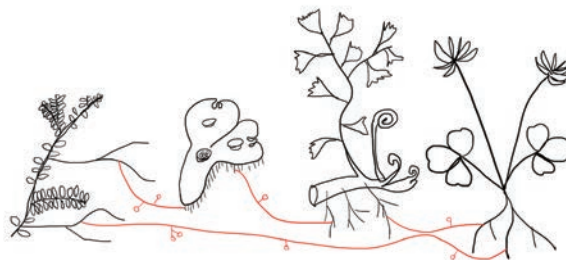


Figure 3. AM fungi form mutualistic symbiosis with most terrestrial plants and can generate superorganisms via their hyphae.

AM fungi are obligate biotrophic fungi that require root colonization to complete a life cycle (Figure 4). Genome analyses of AM fungi demonstrate that they lack several important metabolic enzymes related to the obligate biotrophy. AM fungi have long been considered unculturable without the host. However, co-culture of the AM fungus *Rhizophagus irregularis* and mycorrhiza-helper bacteria—isolates of *Paenibacillus validus*—demonstrated that AM fungi can complete their life cycle in the absence of host plants.

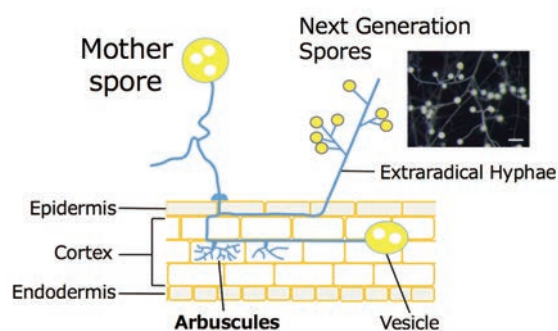


Figure 4. Life cycle of AM fungi. Next-generation spores are formed by root colonization.

Recently, fatty acids have been shown to boost AM fungal growth and sporulation under asymbiotic conditions. Palmitoleic acid and a methyl branched-chain fatty acid isolated from *P. validus* asymbiotically induced infection-competent secondary spores of *R. irregularis*. Further, myristate initiated the asymbiotic growth of AM fungi and can also serve as a carbon and energy source. These findings may lead to the development of new research tools for AM studies and novel generation system of AM fungal inoculants. However, at present, fungal biomass and spore production in the asymbiotic culture systems remain lower than

those in symbiotic co-cultures. Moreover, spores induced by palmitoleic acid or myristate were smaller than those generated symbiotically and their performance as inoculants are unknown.

Not only nutrients but also signalling molecules from host plants may be crucial for AM fungal growth and reproduction. Some phytohormones show positive effects on interactions between AM fungi and hosts. Strigolactone is a major plant-derived signal known to induce hyphal branching and elongation of AM fungi and to stimulate their mitochondrial activity in the pre-symbiotic stage. Methyl jasmonate (MeJA) was increased during AM fungal colonization in roots, consistent with the up-regulation of jasmonic acid biosynthesis genes in plant cortical cells containing arbuscules, highly branched fungal structures for nutrient exchange. Most research of these and other phytohormones are focused on cell-level interactions between AM fungi and plants, and the direct effect of phytohormones on AM fungal growth and reproduction is largely unclear.

We focused on the effect of two phytohormones, strigolactone and MeJA, on AM fungal growth and sporulation in asymbiotic culture supplemented with potassium myristate. We used *R. clarus* HR1 isolated from Hazu-cho, Nishio City, Aichi Prefecture (Figure 5) and found that hundreds of times more secondary spores were produced in medium containing the these two phytohormones than in media without phytohormones in *R. clarus* asymbiotic culture. Furthermore, we confirmed that asymbiotically-produced spores can be sub-cultured and facilitates the growth of plants. Based on these findings, we considered the biological potential of asymbiotically-produced spores.



Figure 5. Spore and hyphae of *R. clarus* HR1.

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### [Original papers]

- Akamatsu, A., Nagae, M., Nishimura, Y., Romero Montero, D., Ninomiya, S., Kojima, M., Takebayashi, Y., Sakakibara, H., Kawaguchi, M., and Takeda, N. (2021). Endogenous gibberellins affect root nodule symbiosis via transcriptional regulation of NODULE INCEPTION in *Lotus japonicus*. *Plant J.* *105*, 1507–1520. DOI: 10.1111/tj.15128
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- Tomoi, T., Kawade, K., Kitagawa, M., Sakata, Y., Tsukaya, H., and Fujita, T. (2020). Quantitative Imaging Reveals Distinct Contributions of SnRK2 and ABI3 in Plasmodesmatal Permeability in *Physcomitrella patens*. *Plant Cell Physiol.* *61*, 942–956. DOI: 10.1093/pcp/pcaa021
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## DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY



Professor  
NIIMI, Teruyuki

Assistant Professor: ANDO, Toshiya  
NAKAMURA, Taro

Technical Staff: MIZUTANI, Takeshi

JSPS Postdoctoral Fellow: SAKAI, Hiroki  
KONAGAYA, Tatsuro  
TAKENAKA, Masaki  
KAWAGUCHI, Haruka

Research Staff: CHIKAMI, Yasuhiko  
SOKENDAI Graduate Student: CHIKAMI, Yasuhiko  
KITAZAWA, Yurina

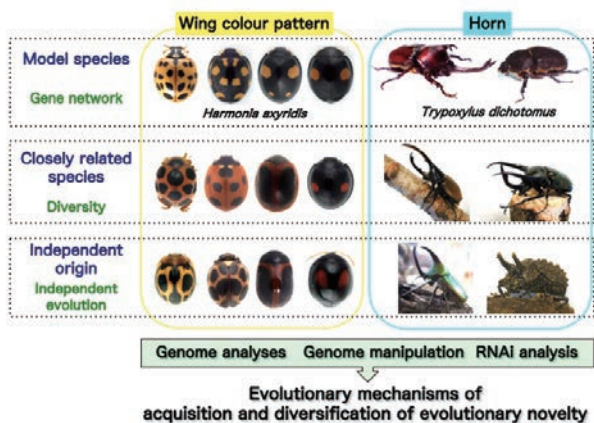
Visiting Scientist: MORITA, Shinichi

Technical Assistant: MORITA, Junko  
HACHISUKA, Yukari  
YOKOYAMA, Michiko  
TAGUCHI, Rie

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 evolution-

ary 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.

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.



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

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.

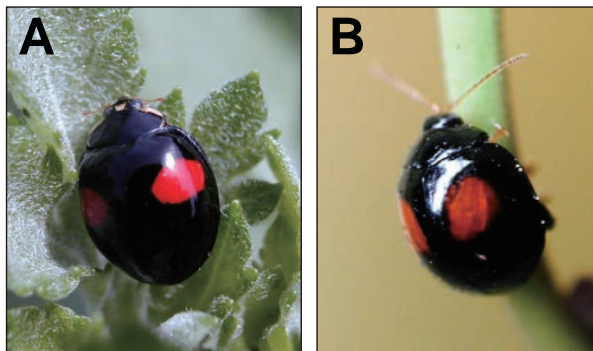


Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

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.

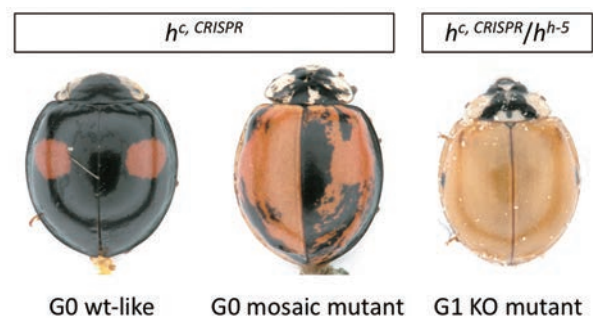


Figure 3. Representative photographs of *pannier* mutant phenotypes in G0 wt-like (left side), G0 mosaic (middle side) and G1 KO mutant (right side).

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 multiple times *de novo*, as evolutionary “novelties”. However, the molecular mechanisms involved in sexually dimorphic horn formation are still poorly understood. To investigate the developmental mechanisms of horn formation, we are focusing on the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns. The male-specific horns of *T. dichotomus* are among the best models for studying how an extreme, sex-specific morphology is formed (Figure 4, Control).

We have recently developed a larval RNAi technique for

*T. dichotomus*, which has allowed us to molecularly dissect the relationship between the conserved genetic pathway for sex differentiation and sexually dimorphic horn formation during post embryonic development. We systematically evaluated the function of the sex determination gene, *transformer (tra)* in different developmental stages, and revealed in which tissue and developmental stage the gene regulatory network for sex differentiation is activated to form sexual dimorphic horns in the head and thorax. In *T. dichotomus*, *tra* regulates sex-specific splicing of the *doublesex* pre-mRNA, and its loss of function results in sex transformation in females (Figure 4). *tra* RNAi treatments in females at early developmental stages during metamorphosis resulted in full sexual transformation, whereas no transformation is observed in the treatments at later stages. Therefore, we were able to estimate the onset of activation of the developmental program for the sexually dimorphic horn formation by determining the latest RNAi treatment timing when a full sexual transformation phenotype is observed. Based on this approach, we estimated that the developmental program for sexually dimorphic horn formation is activated at 29 hours after the prepupal period.

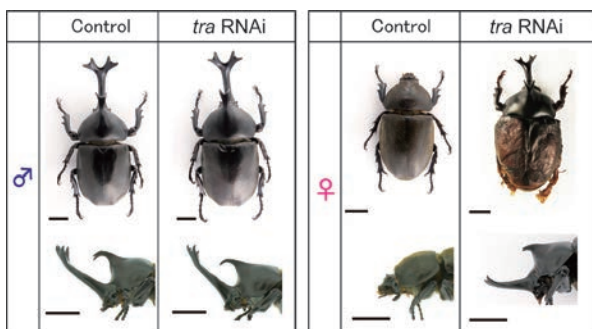


Figure 4. *tra* RNAi phenotypes. In the *tra* RNAi females, ectopic horn formation was caused by sex transformation (masculinization). (Adapted from Morita *et al.*, PLOS Genet., 15: e1008063, 2019)

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

#### Publication List:

##### [Original papers]

- Adachi, H., Matsuda, K., Niimi, T., Kondo, S., and Gotoh, H. (2020). Genetical control of 2D pattern and depth of the primordial furrow that prefigures 3D shape of the rhinoceros beetle horn. *Sci. Rep.* 10. DOI: 10.1038/s41598-020-75709-y
- Chikami, Y., Kawaguchi, H., Suzuki, T., Yoshioka, H., Sato, Y., Yaginuma, T., and Niimi, T. (2021). Oral RNAi of *diap1* results in rapid reduction of damage to potatoes in *Henosepilachna vigintioctopunctata*. *J. Pest Sci.* 94, 505–515. DOI: 10.1007/s10340-020-01276-w
- Sakai, H., Konagaya, T., Takemura, Y., Sahara, K., and Niimi, T. (2020). Double-copulated introduction of ejaculate with dominant larval phenotype to maintain *Bombyx mori* mutant with dysfunctional apyrene sperm. *J. Insect Biotechnol. Sericology* 89, 2\_039-2\_043. DOI: 10.11416/jibs.89.2\_039



## LABORATORY OF EVOLUTIONARY GENOMICS



Professor:  
SHIGENOBU, Shuji

Assistant Professor: HUANGH, Jia-Hsin  
Specially Appointed Assistant Professor:

YODA, Shinichi

Postdoctoral Fellow: KOBAYASHI, Yuuki  
CHUNG, Chen-yo

JSPS Postdoctoral Fellow: NOZAKI, Tomonari  
MATSUDA, Naoki

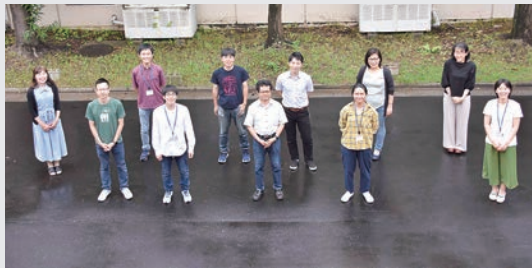
SOKENDAI Graduate Student: YORIMOTO, Shunta  
TAN, Kathrine

Visiting Graduate Student: TONG, Xin

Technical Assistant: SUZUKI, Miyuzu  
WEN, Hsin-I

IKEDA, Mika

Admin Support Staff: ICHIKAWA, Mariko



## Symbiogenomics

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

Every creature on Earth exists among a network of various biological interactions. For example, many multicellular organisms, including humans, harbor symbiotic bacteria in their bodies. Some of them provide their hosts with essential nutrients deficient in the host’s diet and others digest foods that are indigestible by the host alone. Despite numerous examples of symbioses and its intriguing outcomes, the genetic and molecular basis underlying these interactions remains elusive. The goal of our group is to establish a new interdisciplinary science known as “Symbiogenomics”, where we aim to understand the network of biological interactions at the molecular and genetic level. To this end, we take advantage of state-of-the-art genomics, such as next-generation sequencing technologies and CRISPR-Cas9 genome editing.



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, *Acyrtosiphon pisum*, in consort with that of bacterial symbiont *Buchnera aphidicola* illustrates the remarkable interdependency between these two organisms (IAGC, PLOS Biol. 2010; Shigenobu *et al.*, Nature. 2000). The genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. Genome analysis revealed that the pea aphid has undergone characteristic gene losses and duplications. The IMB antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids in obtaining beneficial symbionts. Lineage-specific gene duplications have occurred in genes over a broad range of functional categories, which include signaling pathways, miRNA machinery, chromatin modification and mitosis. The importance of these duplications for symbiosis remains to be determined. We found several instances of lateral gene transfer from bacteria to the pea aphid genome. Some of them are highly expressed in bacteriocytes.

We recently discovered a novel class of genes in the pea aphid genome that encode small cysteine-rich proteins with secretion signals that are expressed exclusively in the bacteriocytes of the pea aphid, and named these bacteriocyte-specific cysteine-rich proteins (BCR) (Shigenobu & Stern, Proc. R. Soc. B 2013). The BCR mRNAs are first expressed at a developmental time point coinciding with the incorporation of symbionts strictly in the cells that contribute to the bacteriocyte, and this bacteriocyte-specific expression is maintained throughout the aphid’s life. Furthermore, some BCRs showed antibiotic activity (Uchi *et al.*, Microbes. Environ. 2019). 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  $\mu$ m.

## Publication List

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### [Original papers]

- Bessho-Uehara, M., Yamamoto, N., Shigenobu, S., Mori, H., Kuwata, K., and Oba, Y. (2020). Kleptoprotein bioluminescence: *Parapriacanthus* fish obtain luciferase from ostracod prey. *Sci. Adv.* 6, eaax4942. DOI: 10.1126/sciadv.aax4942
- Fukutomi, Y., Kondo, S., Toyoda, A., Shigenobu, S., and Koshikawa, S. (2021). Transcriptome analysis reveals wingless regulates neural development and signaling genes in the region of wing pigmentation of a polka-dotted fruit fly. *FEBS J.* 288, 99–110. DOI: 10.1111/febs.15338
- Tominaga, T., Yamaguchi, K., Shigenobu, S., Yamato, M., and Kaminaka, H. (2020). The effects of gibberellin on the expression of symbiosis-related genes in Paris-type arbuscular mycorrhizal symbiosis in *Eustoma grandiflorum*. *Plant Signal. & Behav.* 15. DOI: 10.1080/15592324.2020.1784544
- Mondal, S.I., Akter, A., Koga, R., Hosokawa, T., Dayi, M., Murase, K., Tanaka, R., Shigenobu, S., Fukatsu, T., and Kikuchi, T. (2020). Reduced genome of the gut symbiotic bacterium “*Candidatus Benitsuchiphilus tojoi*” provides insight into its possible roles in ecology and adaptation of the host insect. *Front. Microbiol.* 11. DOI: 10.3389/fmicb.2020.00840
- Li, Y., Omori, A., Flores, R.L., Satterfield, S., Nguyen, C., Ota, T., Tsurugaya, T., Ikuta, T., Ikeo, K., Kikuchi, M., *et al.* (2020). Genomic insights of body plan transitions from bilateral to pentamer symmetry in Echinoderms. *Commun. Biol.* 3. DOI: 10.1038/s42003-020-1091-1
- Vu, T.-D., Lwasaki, Y., Shigenobu, S., Maruko Akiko and Oshima, K., Lioka, E., Huang, C.-L., Abe T., Tamaki, S., Lin, Y.-W., Chen, C.-K., Lu Mei-Yeh and Hojo, M., *et al.* (2020). Behavioral and brain-transcriptomic synchronization between the two opponents of a fighting pair of the fish *Betta splendens*. *PLoS Genet.* 16. DOI: 10.1371/journal.pgen.1008831
- Cui, S., Kubota, T., Nishiyama, T., Ishida, J.K., Shigenobu, S., Shibata, T.F., Toyoda, A., Hasebe, M., Shirasu, K., and Yoshida, S. (2020). Ethylene signaling mediates host invasion by parasitic plants. *Sci. Adv.* 6. DOI: 10.1126/sciadv.abc2385

## LABORATORY OF BIORESOURCES



Specially Appointed Professor  
NARUSE, Kiyoshi

**Research Staff:** KANEKO, Hiroyo  
HARA, Ikuyo  
YANOAWA, Azusa

**Visiting Scientist:** SATO, Tadashi

**Visiting Graduate Student:** IMAEDA YASSUMOTO,  
Tamiris

**Technical Assistant:** AJIOKA, Rie  
KOIKE, Chieko  
KOIKE, Yukari  
TAKAKI, Chikako  
TESHIMA, Yuuko  
TORII, Naoko  
YAMAZAKI, Toko

**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 accessibility 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. Dynamic transcriptional and chromatin accessibility landscape during medaka development.

A high-quality genome sequence and a variety of genetic tools are available for medaka. However, existing genome

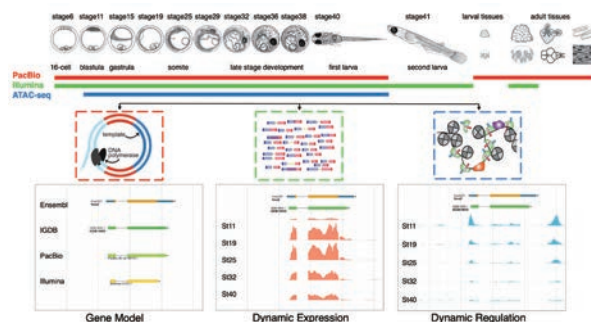


Figure 1. Overview of medaka multi-omics analysis during development

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](http://tulab.genetics.ac.cn/medaka_omics)) will serve as a daily reference tool for the entire medaka community.

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

The body coloration of animals is due to pigment cells derived from neural crest cells which are multipotent and differentiate into diverse cell types. Medaka possess four distinct types of pigment cells known as melanophores, xanthophores, iridophores, and leucophores. The few melanophore (*fm*) mutant found amongst medaka is characterized by reduced numbers of melanophores and leucophores. We have identified kit-ligand as the gene whose mutation gives rise to the *fm* phenotype. This identification was confirmed by generation of kit-ligand knockout medaka and the findings that these fish also manifest reduced numbers of melanophores and leucophores and fail to rescue the *fm* mutant phenotype. We also found that expression of *sox5*, *pax7a*, *pax3a*, and *mitfa* genes is down-regulated in both *fm* and *kit-ligand* knockout medaka, implicating c-Kit signaling in the regulation of the expression of these genes as well as the encoded transcription factors in pigment cell specification.

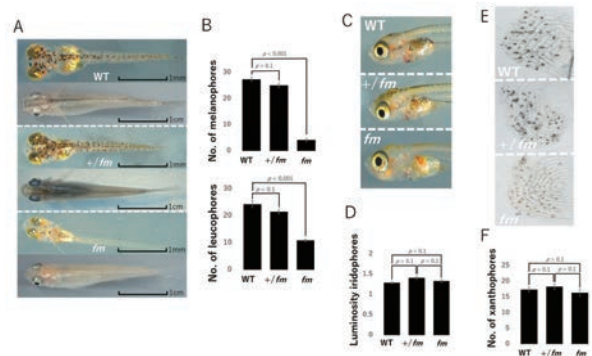


Figure 2. The *fm* mutants show a reduction in melanophore and leucophore pigmentation (A and B) and not in xanthophore and iridophore pigmentation (C, D, E and F).

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

Medaka fish in the genus *Oryzias* are an emerging model system for studying the molecular basis of vertebrate evolution. This genus contains approximately 35 species and exhibits great morphological, ecological and physiological differences among its 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 3. NBRP Medaka website

#### Publication List:

##### (Original papers)

- Ansay, 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., *et al.* (2021). Genome editing reveals fitness effects of a gene for sexual dichromatism in Sulawesian fishes. *Nat. Commun.* 12. DOI: 10.1038/s41467-021-21697-0
- Li, Y., Liu, Y., Yang, H., Zhang, T., Naruse, K., and Tu, Q. (2020). Dynamic transcriptional and chromatin accessibility landscape of medaka embryogenesis. *Genome Res.* 30, 924–937. DOI: 10.1101/gr.258871.119
- Takehana, Y., Zahm, M., Cabau, C., Klopp, C., Roques, C., Bouchez, O., Donnadieu C., Barrachina, C., Journot, L., Kawaguchi, M., Yasumasu, S., *et al.* (2020). Genome sequence of the euryhaline javafish medaka, *Oryzias javanicus*: A small aquarium fish model for studies on adaptation to salinity. *G3: Genes Genomes Genet.* 10, 907–915. DOI: 10.1534/g3.119.400725

Associate Professor  
KODAMA, Ryuji

Visiting Scientist: YOSHIDA, Akihiro

The aim of this laboratory is to observe the variety of morphogenetic processes that occur during the ontogenesis of multicellular organisms and to analyze the mechanisms of such processes through mainly morphological methods. The accumulation of such analyses of the embryogenetic processes of related species is expected to provide an insight into the evolution of morphogenetic processes. This laboratory uses the wings of lepidopteran insects (butterflies and moths) as the main subject of morphological studies.

### I. Wing outline shape formed by cell death

The wings of lepidopteran insects develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. The outline shape of an adult wing often differs from that of a pupal wing. This difference is brought about by the programmed cell death of the marginal area of the pupal wing. The marginal dying area is called “the degeneration region” and the internal area which develops into the adult wing is called “the differentiation region”.

Cell death in the degeneration region proceeds very rapidly and finishes in a half-day to one-day period in *Pieris rapae* and in several other examined species. It has been shown that the dying cells in the degeneration region have characteristics in common with apoptotic cell death in mammalian cells, such as fragmented and condensed nuclei containing short DNA fragments detected by TUNEL staining. The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. The macrophages are excluded from the differentiation region by a close adherence of dorsal and ventral epithelia and concentrated in the degeneration region, thus accelerating the removal of dead cells and the shrinkage of the degeneration region.

A possible physiological role of cell degeneration at the wing margin is to make space for the growth of the marginal scales. These scales are remarkably elongated and grow densely on the edge of the wing. These scales are considered to be important in stabilizing the turbulence occurring posterior to the wing. The movements of the marginal scales are closely monitored by sensory scales and the bristles growing among them.

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

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

concomitant with programmed cell death at the wing margin, and gives rise to the space between the pupal wing and the cuticle. This space appears to allow the growth of long marginal scales.

Microscopic observation of the long marginal scales of *Phthorimaea operculella* have revealed that they have a novel branching morphology and the branches were ubiquitously and densely distributed within the scale array to form a mesh-like architecture similar to a nonwoven fabric. The marginal scales maintain a coherent sheet-like structure during wingbeat (Yoshida, A. *et al.* *Sci. Nat.* 104, 27, 2017).

### III. Transparent wing formation through scale removal

Butterfly and moth wings are usually fully covered with scales. However, in some species, the adult wing lacks scales in some parts, thus giving the wing a transparent appearance.

We studied the emergence process of the *Cephonodes hylas* moth and found that:

- 1) the future transparent part of the wing immediately after emergence is covered with scales that are morphologically different from the scales on the other part
- 2) young adults detach numerous scales on the future transparent part at the initial take-off after emergence, and consequently the transparent part appears.

We also studied the scale morphology and found that the detaching scale is remarkably large and its proximal pedicel, through which the scale attaches to the wing, is tapered (Figure 1). We concluded that these morphological features facilitate the scale detachment through fluttering. (Yoshida, A. *et al.*, Programmed scale detachment in the wing of the pellucid hawk moth, *Cephonodes hylas*: Novel scale morphology, scale detachment mechanisms, and wing transparency. *Zool. Sci.* 38, 427-435, 2021. doi: 10.2108/zs210031).

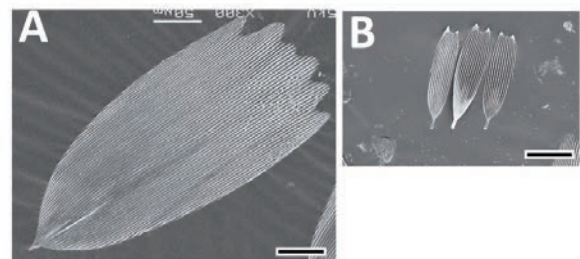
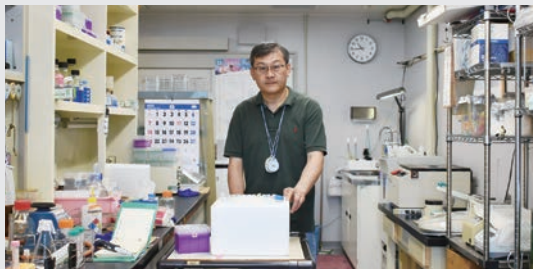


Figure 1. Scanning electron microscopy of the wing scales in the future transparent part (A) and the other part left covered with scales (B) at the same magnification. The scale in A is much larger than that in B, and the pedicel, the protrusion seen at the lower left corner of A is tapered while pedicels in B are columnar. Scale bars: 50 $\mu$ m.

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).

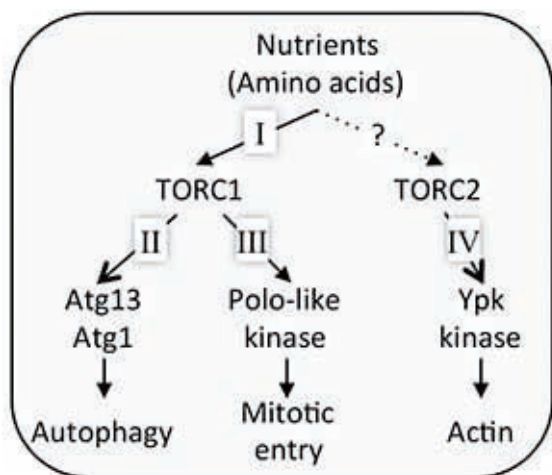


Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

**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).

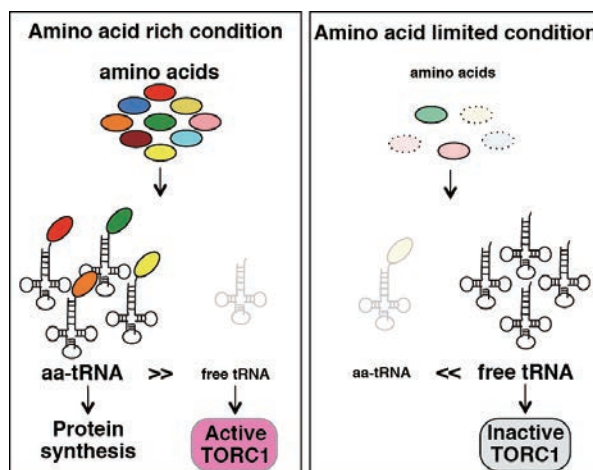


Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

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 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-eIF4E binding was not affected by TORC1 activity, suggesting that the function of the yeast 4EBP is different from that of its mammalian counterparts.

#### Publication List:

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[Original paper]

- Otsubo, Y., Kamada, Y., and Yamashita, A. (2020). Novel links between TORC1 and traditional non-coding RNA, tRNA. *Genes 11*. DOI: 10.3390/genes11090956

## LABORATORY OF BIOLOGICAL DIVERSITY

## HOSHINO Group

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



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

### I. Flower pigmentation patterns

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

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

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

NBRP Morning Glory (described below) maintains approximately 3,500 lines, including two standard lines: Tokyo Kokei Standard and Violet. A high-quality draft whole-genome sequence of Tokyo Kokei Standard, which is accessible from our database, has been previously publicized. Whole genome sequencing of 100 representative lines were performed with the support of NBRP Genome Information Upgrading Program. The lines were chosen to meet the needs of the research community, and to provide information regarding the polymorphisms and gene mutations in the lines. This includes the multiple mutants called ‘Henka-Asagao’ covering the major mutations, and the wild-type plants isolated from natural populations outside of Japan. As a result, total 25,000 *Tpn1* transposon-induced insertion polymorphisms and SNPs, Indels, and CNVs at 25 million loci were discovered.



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, and their phenotypes can be stably inherited by further generations.

Apart from the resequencing analysis, *de novo* genome sequencing for the lines Violet and Africa as well as the *duskish* mutant shown in Figure 1 was performed using HiFi sequencing on a single-molecule real-time sequencer (Sequel II system). The obtained assembly sequences were of equal or better quality than the genome sequence previously reported, and were compiled into a database by the NIBB Data Integration and Analysis Facility to be made available to the public.

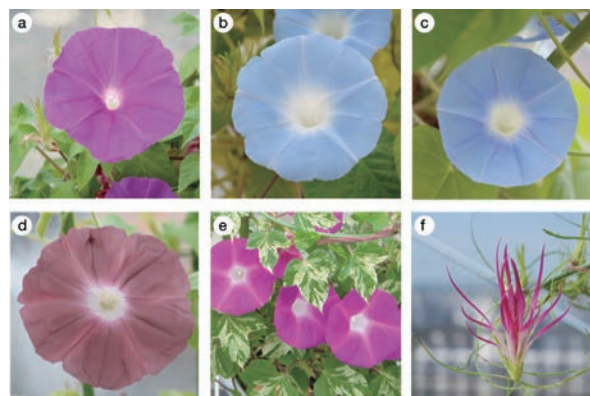


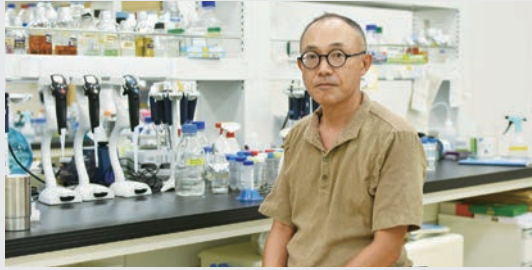
Figure 2. The *I. nil* lines used for the whole-genome analysis. (a) Violet: The most widely used standard line. (b) Africa: The wild-type line isolated in Guinea in 1956. (c) Nepal: The wild-type line isolated by Dr. Sasuke Nakao in Nepal in 1952. (d) Danjuro, one of the most popular cultivars. It was 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 doubled flowers with narrow flower petals and sepals.

### III. Morning glory bio-resources

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glories. In this project, we are collecting, maintaining and distributing standard and mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* is one of the most popular floricultural plants in Japan, and has a 100-year history of extensive genetic studies related to it. Our collection includes 230 lines and 177,000 DNA clones. The whole genome sequence, the transcriptome sequences, as well as the end sequences of the DNA clones can be viewed via the *I. nil* genome database (<http://viewer.shigen.info/asagao/index.php>).

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 nDart1 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.

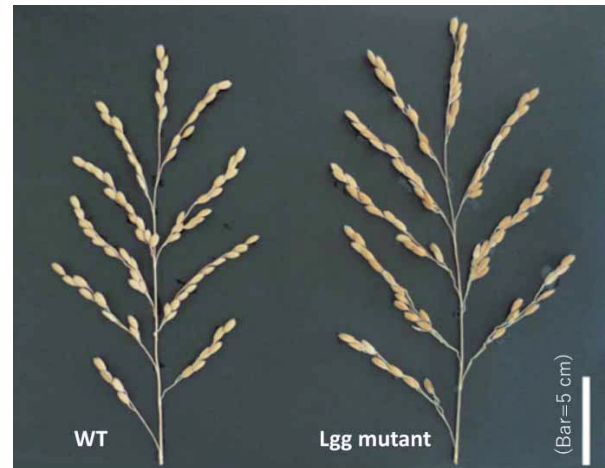


Figure 1. Phenotype of Large gain (*Lgg*). Harvested panicle and seeds.

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 (Figure 2). These results suggest that LGG is localized to the nucleus.

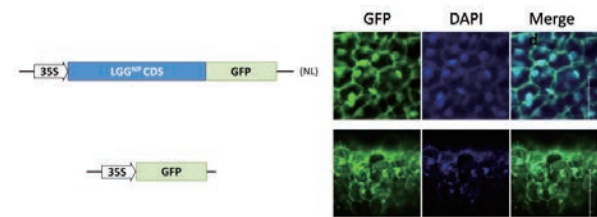


Figure 2. Subcellular localization of LGG. 35S:LGG<sup>NP</sup>-GFP is expressed in rice calli. 4',6-Diamidino-2-phenylindole is used for nuclear staining. Bar = 20 μm.



**LABORATORY OF BIOLOGICAL DIVERSITY**

**KATO Group**

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



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

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

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

**I. 4D cell segmentation/tracking system**

Epithelial morphogenesis in developing embryos is considered to be an important model for collective cell migrations. Drastic cell rearrangements lead to drastic structural changes in building elaborate organs such as the tubular network of *Drosophila* trachea. 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

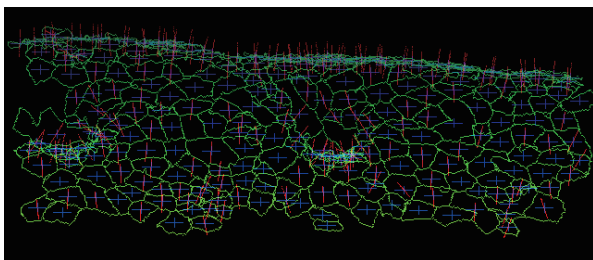


Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

experimental conditions to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system (Shinoda *et al.*).

**II. Image processing pipeline for 3D cell culture**

To elucidate the relationship between mechanical forces underlying the tissue deformation out of large-scale imaging data, we have developed an image processing pipeline for 3D+T imaging datasets.

This pipeline is able to automate a segmentation/quantification process for a large number of images acquired under several experimental conditions for subsequent statistical analysis in addition to building a database of acquired quantities as its final output.

**III. Software for manual image quantification**

Biologically significant imaging features are not always significant to computational algorithms due to their structural instability. This level of difficulty requires inspection conducted by human eyes to extract features from the images gleaned. To simplify this, we have developed a GUI (Graphical User Interface) application which can easily visualize 4D imaging data and has made manual feature annotations easier (Figure 2).

This application is freely available at our website (<https://bioimageanalysis.jp/>).

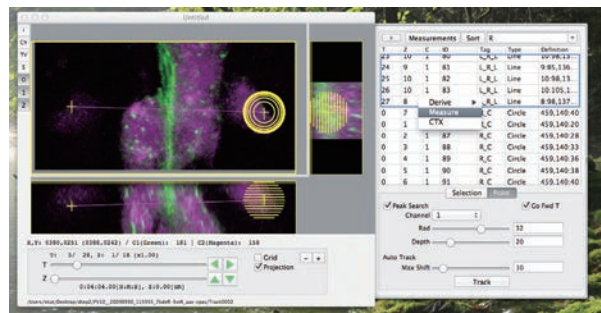


Figure 2. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

**Publication List:**

**[Original papers]**

- Kondrychyn, I., Kelly, D.J., Carretero, N.T., Nomori, A., Kato, K., Chong, J., Nakajima, H., Okuda, S., Mochizuki, N., and Phng, L.-K. (2020). Marcks11 modulates endothelial cell mechanoreponse to haemodynamic forces to control blood vessel shape and size. *Nat. Commun. 11*. DOI: 10.1038/s41467-020-19308-5
- Kurihara, M., Kato, K., Sanbo, C., Shigenobu, S., Ohkawa, Y., Fuchigami, T., and Miyanari, Y. (2020). Genomic profiling by ALaP-Seq reveals transcriptional regulation by PML Bodies through DNMT3A exclusion. *Mol. Cell* 78, 493+. DOI: 10.1016/j.molcel.2020.04.004
- Sakai, Y., Kato, K., Koyama, H., Kuba A., Takahashi, H., Fujimori, T., Hata, M., Negri, A.P., Baird, A.H., and Ueno, N. (2020). A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, *Acropora tenuis*. *Sci. Rep. 10*. DOI: 10.1038/s41598-020-74649-x
- Uemura, Y., Kato, K., Kawakami, K., Kimura Yukiko and Oda, Y., and Higashijima, S. (2020). Neuronal Circuits That Control Rhythmic Pectoral Fin Movements in Zebrafish. *J. Neurosci. 40*, 6678–6690. DOI: 10.1523/JNEUROSCI.1484-20.2020

Specially Appointed Assistant Professor:  
OHTA, Yusaku

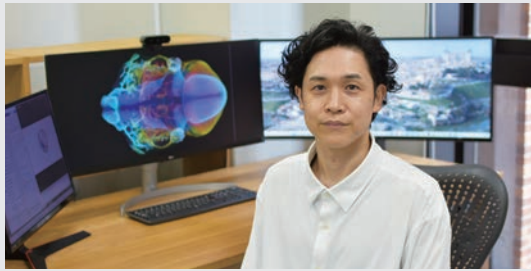


Image analysis is an important element in the understanding of life science. It makes the quantification of phenomena through the extraction of meaningful information from a large amount of images, and the appropriate expression of those information, possible. To this end, I have been developing image analysis technology aimed at analyzing the developmental process of the early zebrafish embryos at a whole embryo scale and single cell resolution. By combining 3D cell tracking and functional imaging, I'm currently developing an analytical technique that can simultaneously extract multiple types of information such as cell morphology, cell motility, and cell dynamics.

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

During early embryogenesis, three-dimensional remodeling of cell populations through cell migration is essential. While individual cell motility depends on extracellular signals, cell-

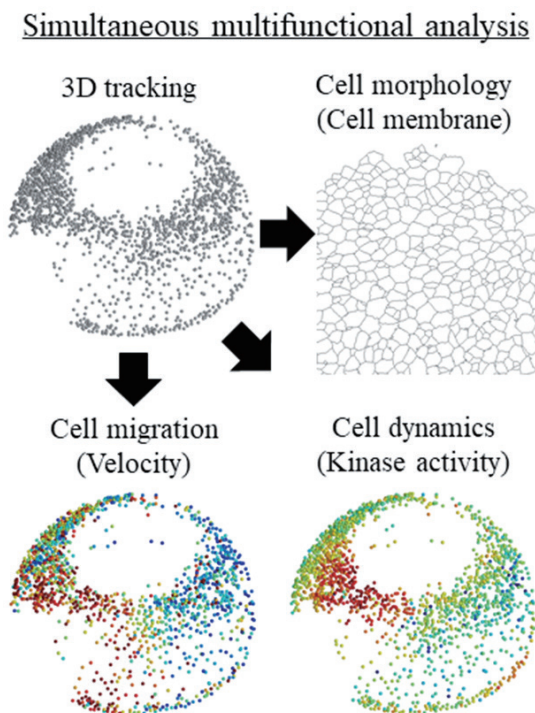


Figure 1. Simultaneous multifunctional analysis of early embryonic development

cell adhesion are able to be maintained, thereby controlling highly coordinated cell motility. To elucidate the principle of such a complicated form of embryogenesis, it is necessary to understand the cell dynamics of the whole embryo at a single cell resolution. To this end, I have been developing image analysis technology to analyze the developmental process of the early zebrafish embryo at a whole embryo scale and single cell resolution.

With the development of microscopic equipment, early embryonic imaging has evolved from two-dimensional, fixed specimens and partial embryonic observations to three-dimensional, live specimens and whole embryo scale. This evolution has led to an explosion in the analysis of cell migration during early embryonic development in recent years. However, conventional image analysis techniques have only been able to extract information on cell migration in early development. Therefore, I have developed an analytic method that can simultaneously extract information concerning cell migration, cell dynamics, and cell morphology at whole embryonic scale and single cell resolution.

### II. Research support by image analysis

The development of imaging technology has been remarkable within life science research, and many researchers are now able to easily acquire large and complex image data sets. However, image analysis can still create hurdles for researchers, as well as bottlenecks in research. In order to solve this problem, I have been providing research support based on the following three concepts.

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

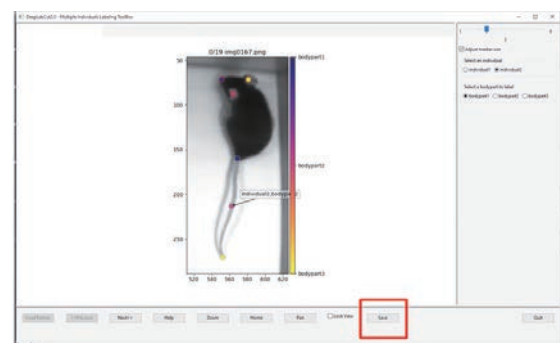


Figure 2. A website that explains how to use image analysis software




**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.

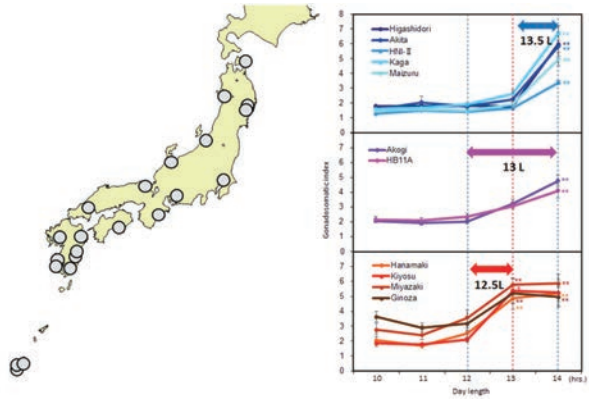


Figure 1. Differences in critical day length (right) were detected among medaka populations that had originated in various latitudes (left).

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<sub>2</sub> 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).

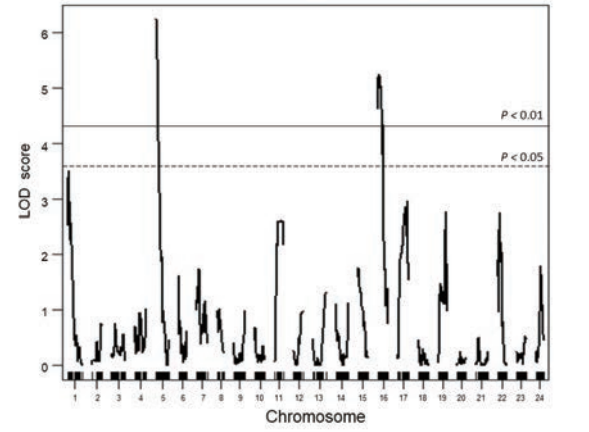


Figure 2. Result of QTL analysis for critical day length.

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:

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##### [Original papers]

- Nakayama, T., Okimura, K., Shen, J., Guh, Y.-J., Tamai, T.K., Shimada, A., Minou, S., Okushi, Y., Shimmura, T., Furukawa, Y., *et al.* (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*. DOI: 10.1371/journal.pone.0234803

## LABORATORY OF BIOLOGICAL DIVERSITY

## SAITO Group

Specially Appointed Associate Professor  
SAITO, Nen

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 sub-cellular scale phenomena as well as cellular and tissue level phenomena.

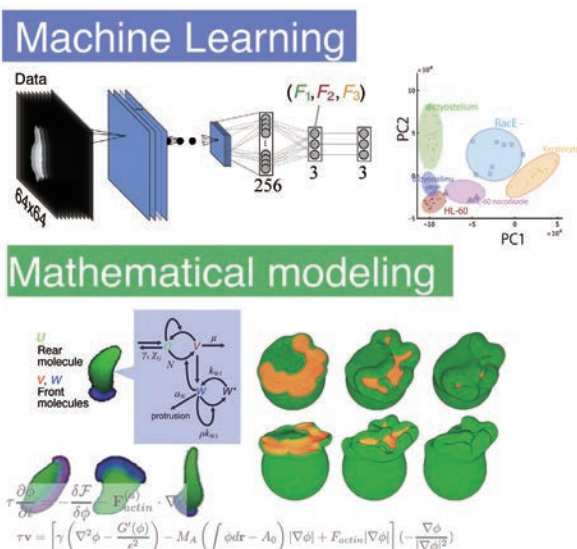


Figure 1. Researches combining machine learning and mathematical modeling.

### 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 respon-

sible for the differences in cell shapes due to cell type and mutation.

We also performed three-dimensional modeling of cell deformation in macropinocytosis, which use actin-dependent endocytosis, and one in which amoeboid cells crawl on structured substrates with an micrometer-scale ridge.

### II. Mathematical Modeling of multicellular systems

In the field of active matter physics, modeling of the collective motions of birds and fish has advanced to the point of revealing how the collective motion of flocking behaviors self-organize according to simple rules. On the other hand, the collective motion of cells differ greatly from flocks of birds or fish in that the shape of individual cells can be drastically deformed. In order to understand phenomena such as the formation, homeostasis, and breakdown of tissues by cell populations, it is very important to study soft deformable active matter that permits deformation while keeping cell motion extremely simple. However, this vein of research is still in its infancy.

Based on the Fourier series expansion of cell contour, we have developed a mathematical model of multicellular systems. This model enables us to simulate the number of deformable cells in the order of thousands to tens of thousands. Using this model, we are now exploring collective phenomena that can emerge from this deformability. Currently, we have discovered that when the deformability of individual cells is reduced, the motion of the entire cell population stalls and they akin to a glass-like material.

### III. 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.

## INTRODUCTION OF THE OPEN LABORATRY

Under the leadership of its Director General, NIBB has been improving research environments in order to strengthen international collaboration, create new biological research fields, and develop truly globalized young researchers from 2019 onwards. As part of this effort, NIBB has established the Open Laboratory: a facility whose establishment was also supported by the National Institutes of Natural Sciences.

Researchers engaged in different research fields come to the Open Laboratory from all around the world to conduct their own research, to be academically stimulated, and to foster interdisciplinary research.

### The basic concepts of the Open Laboratory

1. Promoting interaction between researchers from different research fields and countries in an open laboratory space as well as a shared office space
2. Sharing experimental equipment, instruments, bioresources, and information among participating researchers
3. Promoting interdisciplinary collaborative research
4. Globalizing young researchers through interactions with researchers affiliated with overseas research institutes
5. Planning symposium, seminar and other events to share knowledge.



Unwalled Open Laboratory space



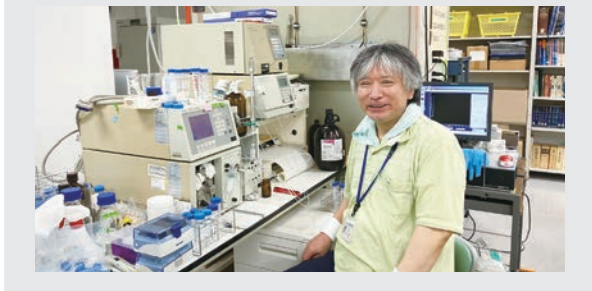
The first Open Laboratory in operation



## LABORATORY OF BIOLOGICAL DIVERSITY

## OHNO Group

Assistant Professor: OHNO, Kaoru



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

### I. Gonadotropins in the starfish, *Patiria pectinifera*

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

The gonad-stimulating substance (GSS) of an echinoderm, the starfish, was the very first gonadotropin to be identified in invertebrates. GSS mediates oocyte maturation in starfish by acting on the ovary to produce the maturation-inducing hormone (MIH), 1-methyladenine, which in turn induces the maturation of the oocytes. In this sense, GSS is functionally identical to vertebrate LH, especially piscine and amphibian LHs, and acts on the ovarian follicle cells to produce MIH to induce the final maturation or meiotic resumption of the oocyte. Considering the functional similarity that GSS shares with vertebrate LH, it is very important from an evolutionary point of view to know the chemical and molecular structure of GSS. We cloned the gene encoding the amino acid sequence of purified GSS from radial nerves of the starfish, *Patiria 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

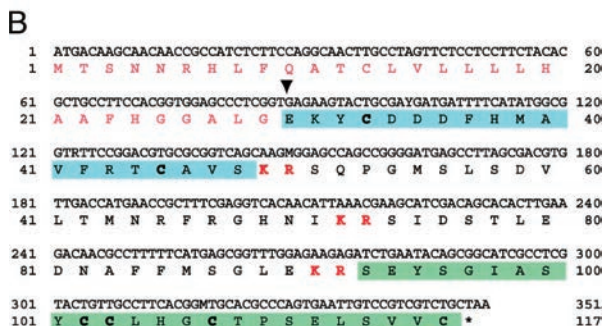


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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 NGIWIY-amide peptide, is in the sea cucumber *Aposticopus japonicus*. We are currently preparing for this research's publication.

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. In particular, relaxin like peptide precursor genes and insulin/IGF like peptide precursor genes were identified from many species. We are producing these neuropeptides 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*

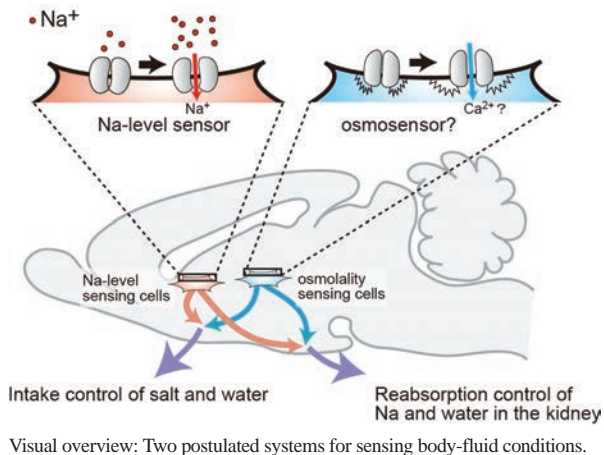
Sequence analysis by capillary sequencer was carried out from the tilling library of *O. latipes* in order to analyze the function of fish female reproductive hormone E2 as a part of our collaborative research. Upon the discovery of aromatase mutants, a detailed analysis was carried out using these strains.

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  $\text{Na}^+$  ( $[\text{Na}^+]$ ) 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  $\text{Na}_x$  and TRPV4

$[\text{Na}^+]$  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  $\text{Na}_x$ , which structurally resembles voltage-gated sodium channels ( $\text{Na}_v1.1-1.9$ ), is the brain  $[\text{Na}^+]$  sensor to detect increases in  $[\text{Na}^+]$  in body fluids.  $\text{Na}_x$  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  $\text{Na}_x$  signals in these brain regions deficient in a blood-brain barrier are involved in the control of salt intake.

We recently demonstrated that  $\text{Na}_x$  signals are also involved in the control of water intake behavior. The signaling mechanisms in the OVLT for water-intake induction by increases in  $[\text{Na}^+]$  in body fluids are presented in Figure 1. When  $[\text{Na}^+]$

in plasma and CSF increases,  $\text{Na}_x$  channels in glial cells in the OVLT are activated, leading to the synthesis of epoxyeicosatrienoic acids (EETs) in  $\text{Na}_x$ -positive glial cells. EETs released from  $\text{Na}_x$ -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.

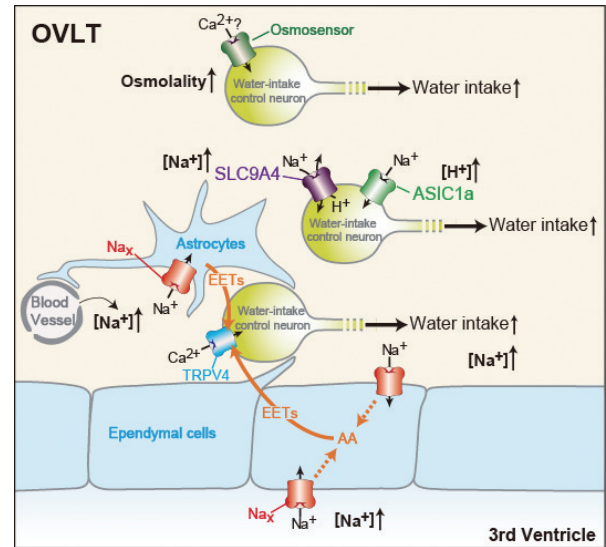


Figure 1. Schematic drawings of sensing mechanisms of body-fluid conditions in the OVLT responsible for the induction of water intake. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

Water intake by  $\text{Na}_x$ -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  $\text{Na}_x$ -KO and WT mice after an ICV injection of an equimolar hypertonic sorbitol solution. These findings suggest the existence of another unknown  $[\text{Na}^+]$  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. This year, we revealed that SLC9A4 in the OVLT functions as a  $[\text{Na}^+]$  sensor for the control of water intake behavior among these candidates, and that the signaling pathway originating from this sensor is independent of the  $\text{Na}_x$ /TRPV4 pathway, another  $[\text{Na}^+]$ -sensing pathway for the control of water intake (Figure 1). Our experimental results suggested that SLC9A4-positive neurons are activated via ASIC1a in a  $\text{H}^+$ -dependent manner. Our experimental results also revealed that water intake induced by the increase in  $[\text{Na}^+]$  in CSF was completely lost in *slc9a4*-knockdown  $\text{Na}_x$ -KO mice. Thus, water intake induced by  $[\text{Na}^+]$  increases in body fluids may be explained by the  $\text{Na}_x$ /TRPV4 and SLC9A4/ASIC1a pathways. In addition to these two  $[\text{Na}^+]$ -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.

**Publication List:**

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**[Original papers]**

- Sakuta, H., Lin, C.-H., Hiyama, T.Y., Matsuda, T., Yamaguchi, K., Shigenobu, S., Kobayashi, K., and Noda, M. (2020). SLC9A4 in the organum vasculosum of the lamina terminalis is a [Na<sup>+</sup>] sensor for the control of water intake. *Pflügers Arch. - Eur. J. Physiol.* 472, 609–624. DOI: 10.1007/s00424-020-02389-y
- Sakuta, H., Lin, C.-H., Yamada, M., Kita Y., Tokuoka, S.M., Shimizu, T., and Noda, M. (2020). Na<sub>x</sub>-positive glial cells in the organum vasculosum laminae terminalis produce epoxyeicosatrienoic acids to induce water intake in response to increases in [Na<sup>+</sup>] in body fluids. *Neurosci. Res.* 154, 45–51. DOI: 10.1016/j.neures.2019.05.006



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 using *Macrostomum lignano*, to test the function of genes shown to be involved in positional control during growth and regeneration. I am currently adapting the infrared laser evoked gene operator (IR-LEGO) technology to use with the previously established HSP20 promoter. This will enable me to track the cell fate in vivo and overexpress selected genes even on a single cell level.

To continue this research, I have been awarded the Mitsubishi Foundation grant.

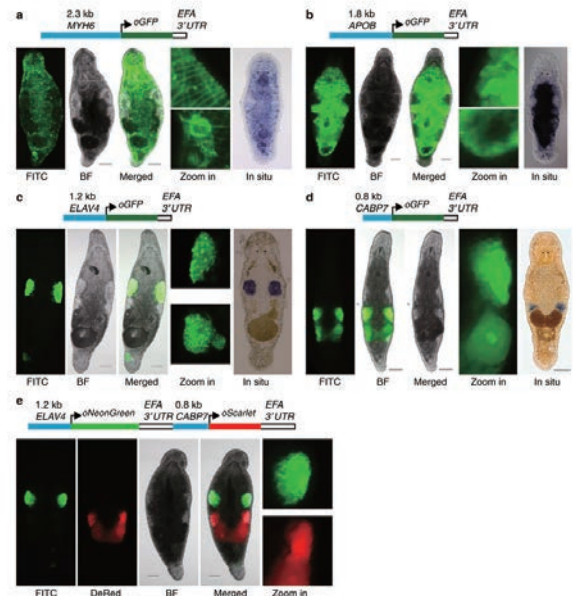


Figure 1. Tissue specific transgenic line of *Macrostomum lignano*.

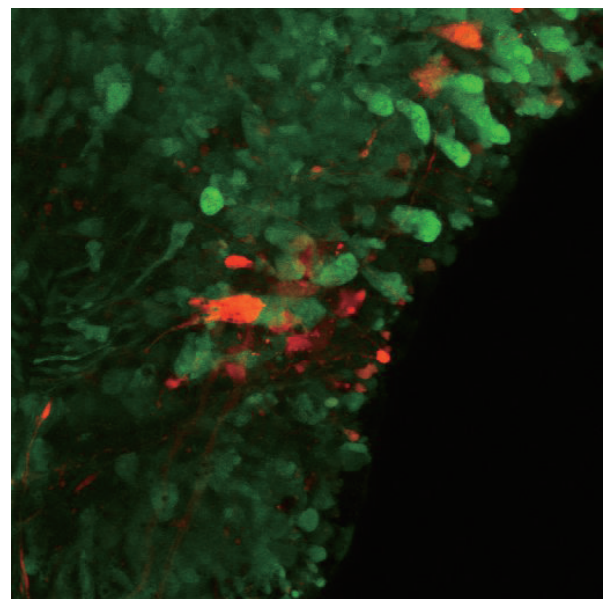
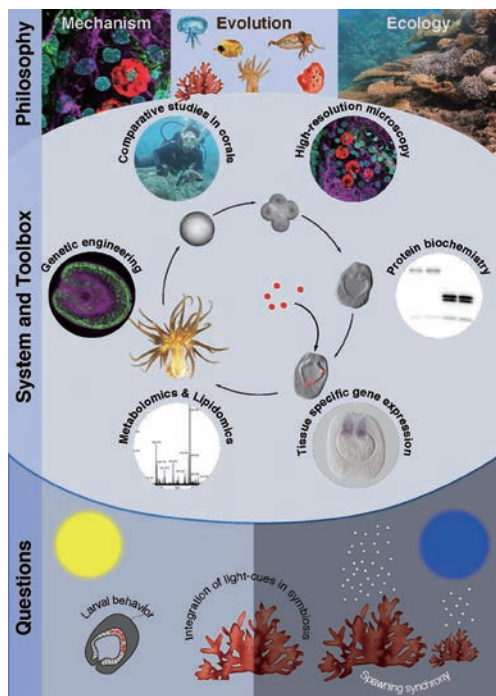


Figure 2. Expression of mScarlet under the HSP20 promoter 24 hours after induction using IR-LEGO

## 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.

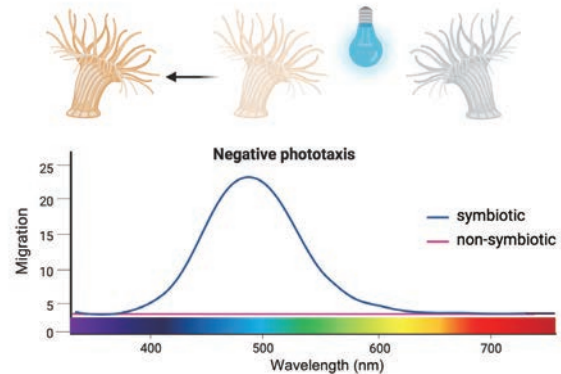


Figure 1. Wavelength specificity of negative phototaxis in symbiotic *Aiptasia*.

## 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 11/18 opsins including the 3 symbiosis-specific ones. Moreover, using the cell culture cAMP signaling assay, we were able to detect a light response for several (7/18) opsins. Determining of their absorbance wavelength specificity is key in relating distinct opsins with light-dependent behavior including phototaxis and spawning in *Aiptasia*.

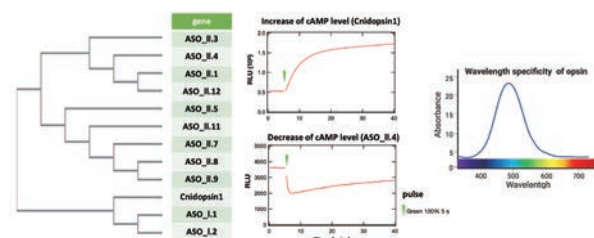


Figure 2. Functional analysis of *Aiptasia* opsins cloned in this study.

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 laboratory, we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis (Figure 1), to research the mechanisms by which cells switch from mitosis (a kind of cell division that divides cells equally to create two identical cells) to meiosis. In addition, we have started to study cellular responses to a novel stress: atmospheric pressure plasma.

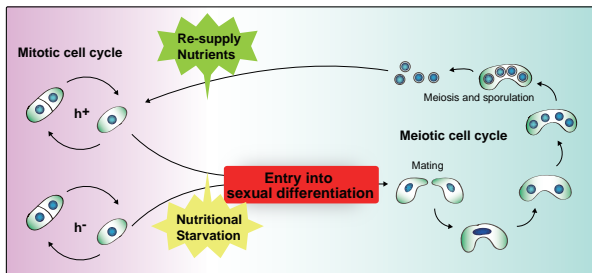


Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting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

I. Signaling pathways that regulate the onset of sexual differentiation

We have been trying to elucidate how *S. pombe* cells switch their cell cycle mode from mitotic to meiotic. To achieve this, we 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 and 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 five of the eight responsible gene encoded tRNA-related factors. The *hmt1* and *hmt2* genes encoded aminoacyl-tRNA synthetases for asparagine and proline, respectively. The *hmt3* gene encodes tRNA adenosine-34 deaminase. The *hmt4* is identical to *rpc34*, which encodes a subunit of RNA polymerase III. The *hmt5* is identical to *sfc4*, which encodes a subunit of the RNA polymerase III-specific general transcription factor IIIC. In the *hmt1-5* mutants, TORC1 activity is downregulated, suggesting that the products of these *hmt* genes may function upstream of TORC1. We also found that 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 starvation.

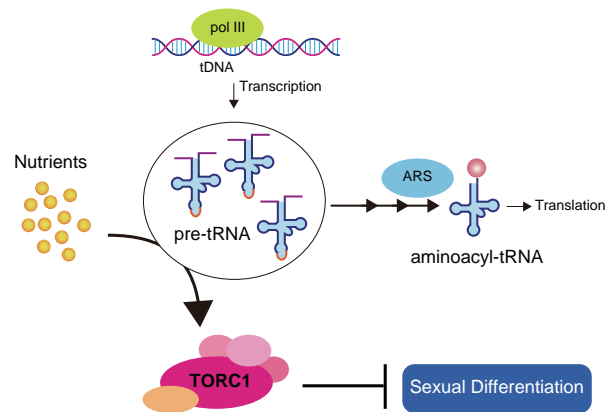


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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 hexa-



nucleotide 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.

### 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.

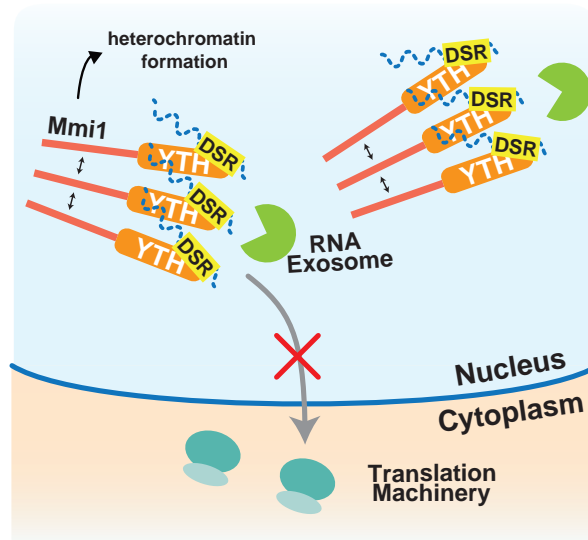


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

#### Publication List:

##### [Original papers]

- Ohtsuka, H., Kobayashi, M., Shimasaki, T., Sato, T., Akanuma, G., Kitaura, Y., Otsubo, Y., Yamashita, A., and Aiba, H. (2021). Magnesium depletion extends fission yeast lifespan via general amino acid control activation. *Microbiologyopen* 10. DOI: 10.1002/mbo3.1176
- Yagi-Utsumi, M., Tanaka, T., Otsubo, Y., Yamashita, A., Yoshimura, S., Nishida, M., and Kato, K. (2021). Cold atmospheric plasma modification of amyloid beta. *Int. J. Mol. Sci.* 22. DOI: 10.3390/ijms22063116

##### [Review Articles]

- Otsubo, Y., Kamada, Y., and Yamashita, A. (2020). Novel links between TORC1 and traditional non-coding RNA, tRNA. *Genes* 11. DOI: 10.3390/genes11090956
- Yoshimura, S., Otsubo, Y., Yamashita, A., and Ishikawa, K. (2020). Insights into normothermic treatment with direct irradiation of atmospheric pressure plasma for biological applications. *Jpn. J. Appl. Phys.* 60, 10502. DOI: 10.35848/1347-4065/abcbcd2

The Astrobiology Center (ABC) was established in 2015 to promote interdisciplinary studies between fields that include astronomy, earth science, and biology. After 5 years of activity, ABC has come to be recognized as the hub institute of astrobiology within Japan. Consequently, this discipline has become established within the Japanese scientific community.

Our ultimate goal is to find a planet akin to a ‘Second Earth’ as well as extraterrestrial life on said planet. Astronomers and earth scientists in the Exo-Planet Search Project and Astrobiology Instrument Project at the main office in Tokyo are now discovering habitable planets around the stars nearest to Earth by using the latest observation technologies. To support these observation projects, three groups from NIBB, the Takizawa, Johzuka, and Fujita groups, are participating in the Exo-Life Search Project to investigate life on Earth utilizing three different approaches: 1) assessing the biosignature of various photosynthetic organisms in order 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.

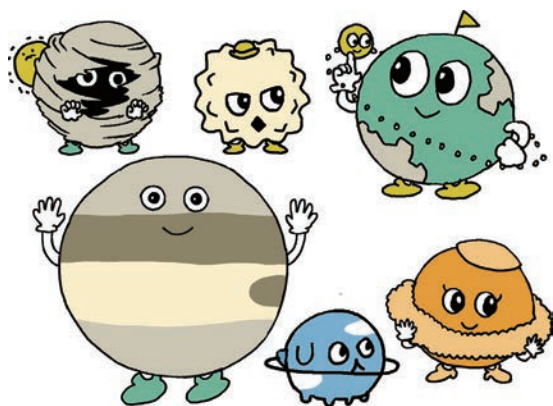


Figure 1. ABC mascot characters: “Exoplanet Friends” (Credit: Hayanon Science Manga Studio)

## 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 ‘Second Earth’.

### Vegetation red-edge of floating plants on ocean planets

One of the most plausible biosignatures on habitable exoplanets is a specific reflection pattern on the land surface named ‘red-edge’ that is caused by land vegetation. Red-edge appears on Earth between red light, which is absorbed by photosynthetic pigments, and near infrared radiation (NIR), which is reflected via leaf tissue structure. While red light absorption is a common feature of all phototrophs, NIR reflection is more noticeable in plants at a higher elevation on land. According to the latest planet formation theory, habitable exoplanets may have too much water to form continents. We have subsequently proposed that there is a possibility that strong biosignatures could be detected on ‘water planets’ due to the extensive flourishing of drifting algae and floating plants. Remote sensing of light reflection by drones revealed that water plants with floating leaves reflect NIR in a manner comparable to land vegetation.

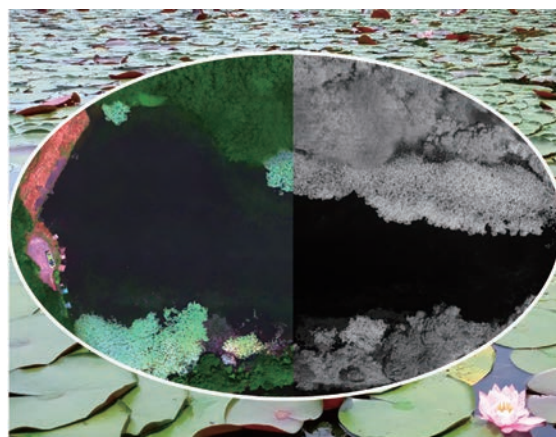


Figure 2. Remote sensing of light reflectance of floating leaf of waterlily. Light reflectance in visible light (BGR image on the left) and NIR (gray scale image on the right) were obtained by the drone-based multiband sensor.

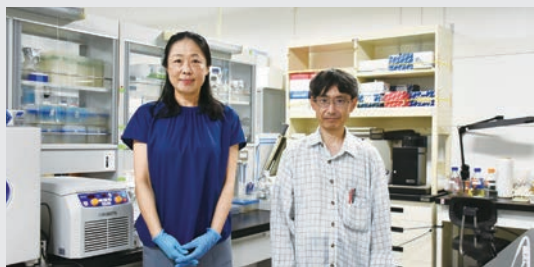
### NIR driven photosynthesis around red dwarfs

Most habitable planets orbit around red dwarfs that are predominantly exposed to NIR. In order to utilize NIR for photosynthesis, alien plants should have NIR-absorbing, photochemically reactive pigments. We have thus evaluated optical and photochemical properties of various potential photosynthetic pigments via quantum chemical calculations, and revealed that several metal-pigments can be functional in reaction centers under NIR radiation conditions.

## 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.

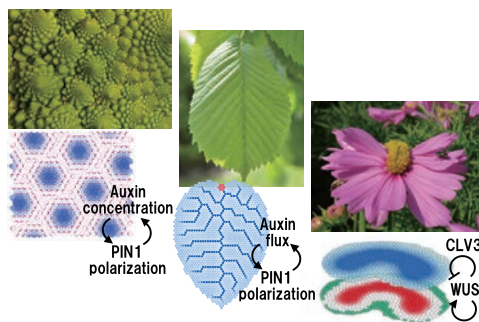


Figure 1. (Left) Phyllotaxis pattern is self-organized by the feedback regulation between auxin concentration and PIN1 polarization. (Middle) Leaf venation pattern is self-organized by the feedback regulation between auxin flux and PIN1 polarization. (Right) Shoot apical meristem (SAM) is self-organized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

## I. Spatiotemporal patterns of cell population

*Escherichia coli* can move towards higher concentrations of nutrients, such as Asp and Ser, by using flagella. Chemotaxis can induce periodic patterns in a self-organizing manner (Figure 2; *Nature* 349, 630–633, 1991). 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 apply the synthetic

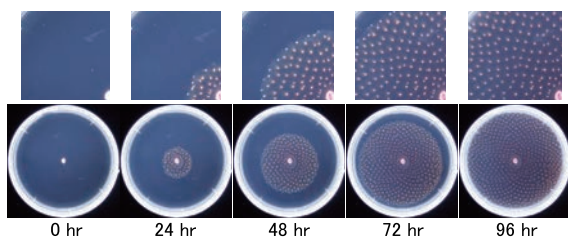


Figure 2. An example of self-organized colony patterns induced by chemotaxis in *E. coli* population.

approach to *E. coli* population to understand the principle of ‘spatiotemporal self-organization of cell populations’.

## II. Spatial regulation of resource allocation in response to nutritional availability

It is critical for living organisms to appropriately allocate resources among its organs, or within a specific organ, because available resources are generally limited. For example, in response to the nutritional environments of their soil, plants regulate resource allocation in their roots in order to plastically change their root system architecture (RSA), so they can efficiently absorb nutrients (Figure 3A). However, it is still not understood why and how RSA is adaptively controlled. Therefore, we modeled and investigated the spatial regulation of resource allocation by focusing on RSA in response to nutrient availability, and provided analytical solutions to the optimal strategy in the case of simple fitness functions (Fujita *et al.*, *J. Theor. Biol.* 486, 110078, 2020). First, we showed that our model could explain the experimental evidence indicating that root growth is maximized at the optimal nutrient concentration under homogeneous conditions. Next, we extended our model to incorporate the spatial heterogeneity of nutrient availability. This extended model revealed that growth suppression by systemic control is required for adaptation to high nutrient conditions, whereas growth promotion by local control is sufficient for adaptation to low-nutrient environments (Figure 3B). This evidence indicates that systemic control can be evolved in the presence of excessive amounts of nutrition, consistent with the ‘N-supply’ systemic signal that is observed experimentally. Furthermore, our model can also explain various experimental results using nitrogen nutrition, and provides a theoretical basis for understanding the spatial regulation of adaptive resource allocation in response to nutritional environment.

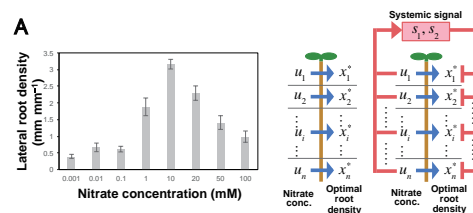


Figure 3. (A) Root growth in the homogeneous availability of nitrogen nutrition. (B) Schematic representation of the spatial regulation of the optimal resource allocation in response to nutrient availability. (right) In the low nutrient availability, the optimal root density is promoted by nutrient through local control (blue arrows). (left) By contrast, in addition to local control, is suppressed through systemic control (denoted in red) under high nutrient availability.

### Publication List:

#### [Original paper]

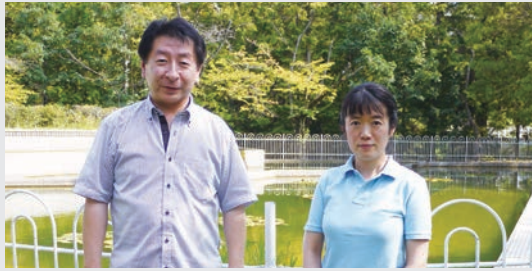
- Kataoka, K., Fujita, H., Isa, M., Gotoh, S., Arasaki, A., Ishida, H., and Kimura, R. (2021). The human *EDAR* 370V/A polymorphism affects tooth root morphology potentially through the modification of a reaction–diffusion system. *Sci. Rep.* 11, 5143. DOI: 10.1038/s41598-021-84653-4



LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: JOHZUKA, Katsuki  
 Technical Staff: ISHINE Naomi



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

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

**I. Dynamic relocalization of condensin during meiosis**

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

densin from rDNA repeat to centromere had occurred. As expected, chromatin-IP experiments indicated that condensin re-localizes to the centromere during meiosis I. Condensin might clamp sister-chromatids together during meiosis I.

**II. Condensin-dependent chromatin folding**

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

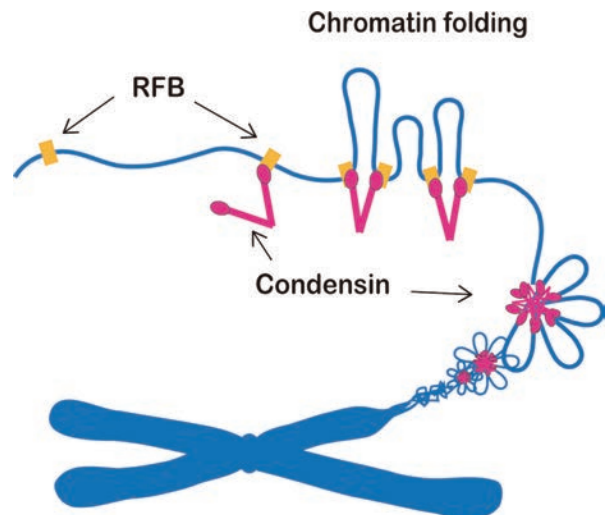


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

**DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY**



Professor  
 MINAGAWA, Jun



Associate Professor  
 TAKAHASHI, Shunichi

Assistant Professor: TOKUTSU, Ryutaro

KIM, Eunuchul

Technical Staff: NODA, Chiyo

Postdoctoral Fellow: SATO, Ryoichi

KAMADA, Konomi

Research Staff: ISHII, Asako

SOKENDAI Graduate Student: WATANABE, Akimasa

OKAJIMA, Keisuke

YANAKA, Ayako

Visiting Scientist: DANN, Marcel

Technical Assistant: YONEZAWA, Harumi

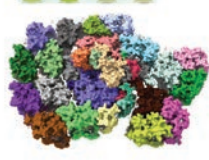
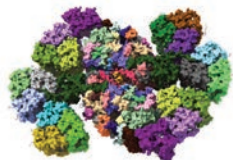
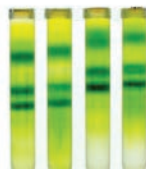
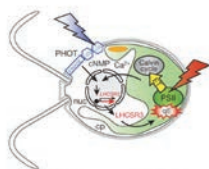
KADOWAKI, Tamaka

Admin Support Staff: TOYAMA, Mami

IIDA, Kaoru



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. Structural characterization of the photosystems in the green alga *Chlorella sorokiniana*.**

The photosynthetic conversion of light energy into chemical energy is performed by photosystems II and I (PSII and PSI) embedded within the thylakoid membranes. In plants and green algae, PSII and PSI comprise the core complex and light-harvesting complexes (LHCII and LHCI), forming PSII-LHCII and PSI-LHCI supercomplexes, respectively. The structural information about photosystem supercomplexes of green algae has been limited to chlorophytic algae. Here, to obtain an insight into the evolution of Chlorophyta, we determined the supramolecular organization of the PSII-LHCII and PSI-LHCI supercomplexes from the freshwater green alga *Chlorella sorokiniana*, which belongs to Trebouxiophyceae (Fig. 1) (Watanabe and Minagawa, *Planta*, 252:79). The obtained results showed that the supramolecular organizations of the photosystem supercomplexes in *C. sorokiniana* were essentially the same as those of the model green alga *C. reinhardtii*, which belongs to Chlorophyceae, namely PSII-LHCII supercomplex formed the C<sub>2</sub>S<sub>2</sub>M<sub>2</sub>L<sub>2</sub> configuration and PSI-LHCI supercomplex was associated with 10 LHCI subunits.

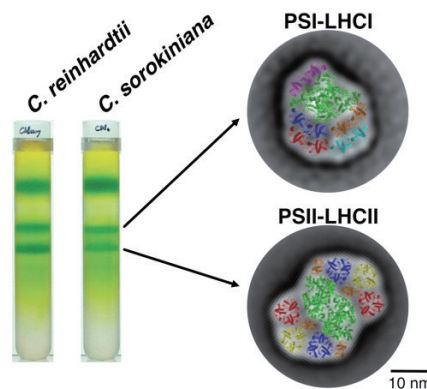


Figure 1. Single-particle EM analysis of photosystems isolated from SDG ultracentrifugation of the  $\alpha$ -DDM-solubilized thylakoids from *C. sorokiniana* cells. Watanabe and Minagawa (2020) *Planta*, 252:79.

**II. Characterization of a giant photosystem I supercomplex in the symbiotic dinoflagellate Symbiodiniaceae.**

Symbiodiniaceae are symbiotic dinoflagellates that provide photosynthetic products to corals. Because corals are distributed across a wide range of depths in the ocean, Symbiodiniaceae species must adapt to various light environments to optimize their photosynthetic performance. However, as few biochemical studies of Symbiodiniaceae photosystems have been reported, the molecular mechanisms of photoadaptation in this algal family remain poorly understood. Here, to investigate the photosynthetic machineries in Symbiodiniaceae, we purified and characterized the PSI supercomplex from the genome-sequenced *Breviolum minutum* (formerly *Symbiodinium minutum*) (Fig. 2) (Kato *et al.*, *Plant Physiol.*, 183:1725-1734). Mass spectrometry analysis revealed 25 light-harvesting complexes (LHCs), including both LHCF and LHCR families, from the purified PSI-LHC supercomplex. Single-particle electron microscopy



showed unique giant supercomplex structures of PSI that were associated with the LHCs. Moreover, the PSI-LHC supercomplex contained a significant amount of the xanthophyll cycle pigment diadinoxanthin. Upon high light treatment, *B. minutum* cells showed increased nonphotochemical quenching, which was correlated with the conversion of diadinoxanthin to diatoxanthin, occurring preferentially in the PSI-LHC supercomplex. The possible role of PSI-LHC in photoprotection in Symbiodiniaceae was discussed.

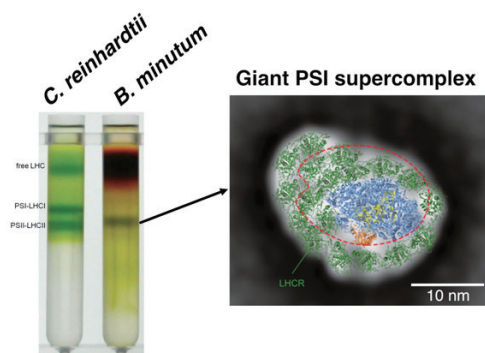


Figure 2. Single-particle EM analysis of photosystem I isolated from SDG ultracentrifugation of the  $\alpha$ -DDM-solubilized thylakoids from *B. minutum* cells. Kato *et al.* (2020) *Plant Physiol.*, 183:1725-1734.

### III. Multimeric and monomeric photosystem II supercomplexes represent structural adaptations to low- and high-light conditions.

An intriguing molecular architecture called the “semi-crystalline photosystem II (PSII) array” has been observed in the thylakoid membranes in vascular plants. It is an array of PSII–LHCII supercomplexes that only appears in low light, but its functional role has not been clarified. Here, we identified PSII–LHCII supercomplexes in their monomeric and multimeric forms in low light–acclimated spinach leaves and prepared them using sucrose-density gradient ultracentrifugation in the presence of amphipol A8-35. When the leaves were acclimated to high light, only the monomeric forms were present, suggesting that the multimeric forms represent a structural adaptation to low light and that disaggregation of the PSII–LHCII supercomplex represents an adaptation to high light. Single-particle EM revealed that the multimeric PSII–LHCII supercomplexes are composed of two (“mega-complex”) or three (“arraycomplex”) units of PSII–LHCII supercomplexes, which likely constitute a fraction of the semi-crystalline PSII array. Further characterization with fluorescence analysis revealed that multimeric forms have a higher light-harvesting capability but a lower thermal dissipation capability than the monomeric form. These findings suggest that the configurational conversion of PSII–LHCII supercomplexes may serve as a structural basis for acclimation of plants to environmental light (Fig. 3) (Kim and Watanabe *et al.*, *J. Biol. Chem.*, 295:14537-14545).

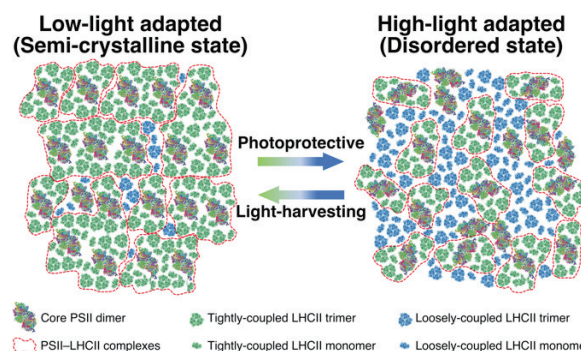


Figure 3. Schematic representation of the putative arrangements of PSII–LHCII supercomplexes in the thylakoid membrane in low-light and high-light conditions. Kim, Watanabe *et al.* (2020) *J. Biol. Chem.*, 295:14537-14545.

### IV. Photoprotective capabilities of light-harvesting complex II trimers in a green alga *Chlamydomonas reinhardtii*.

Major light-harvesting complex (LHCII) trimers in plants induce the thermal dissipation of absorbed excitation energy against photooxidative damage under excess light conditions. LHCII trimers in green algae have been thought to be incapable of energy dissipation without additional quencher proteins, although LHCII in plants and green algae are homologous. In this study, we investigated the energy-dissipative capabilities of four distinct types of LHCII trimers isolated from the model green alga *Chlamydomonas reinhardtii* using spectroscopic analysis. Our results revealed that the LHCII trimers possessing LHCII type II (LHCBM5) and LHCII type IV (LHCBM1) had efficient energy-dissipative capabilities, whereas LHCII type I (LHCBM3/4/6/8/9) and type III (LHCBM2/7) did not. On the basis of the amino acid sequences of LHCBM5 and LHCBM1 compared with the other LHCBMs, we propose that positively charged extra N-terminal amino acid residues mediate the interactions between LHCII trimers to form energy-dissipative states (Fig. 4) (Kim *et al.*, *J. Phys. Chem. Lett.*, 11:7755-7761).

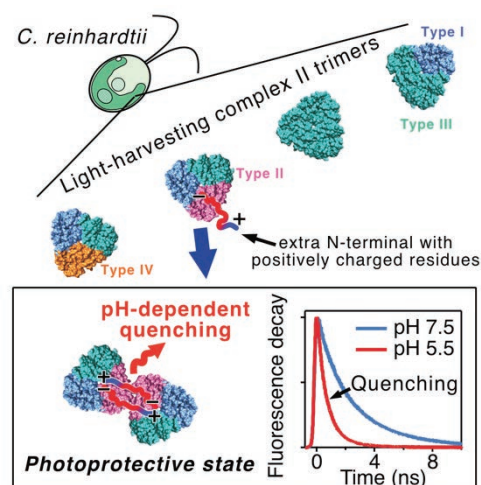


Figure 4. Schematic representation of the extra N-terminal-dependent photoprotective states of LHCII trimers from *C. reinhardtii*. Kim *et al.* (2020) *J. Phys. Chem. Lett.*, 11:7755-7761.



## Publication List:

### [Original Papers]

- Faktorova, D., Nisbet, R.E.R., Robledo, J.A.F., Casacuberta, E., Sudek, L., Allen, A.E., Ares Jr., M., Areste, C., Balestreri, C., Barbrook, A.C., *et al.* (2020). Genetic tool development in marine protists: emerging model organisms for experimental cell biology. *Nat. Methods* 17, 481–494. DOI: 10.1038/s41592-020-0796-x
- Kato, H., Tokutsu, R., Kubota-Kawai, H., Burton-Smith, R.N., Kim, E., and Minagawa, J. (2020). Characterization of a giant PSI Supercomplex in the symbiotic dinoflagellate symbiodiniaceae. *Plant Physiol.* 183, 1725–1734. DOI: 10.1104/pp.20.00726
- Kim, E., Kawakami, K., Sato, R., and Ishii A., and Minagawa, J. (2020). Photoprotective capabilities of light-harvesting complex II trimers in the green alga *Chlamydomonas reinhardtii*. *J. Phys. Chem. Lett.* 11, 7755–7761. DOI: 10.1021/acs.jpcllett.0c02098
- Kim, E., Watanabe, A., Duffy, C.D.P., Ruban, A. V, and Minagawa, J. (2020). Multimeric and monomeric photosystem II supercomplexes represent structural adaptations to low- and high-light conditions. *J. Biol. Chem.* 295, 14537–14545. DOI: 10.1074/jbc.RA120.014198
- Kishimoto, M., Baird, A.H., Maruyama, S., Minagawa, J., and Takahashi, S. (2020). Loss of symbiont infectivity following thermal stress can be a factor limiting recovery from bleaching in cnidarians. *ISME J.* 14, 3149–3152. DOI: 10.1038/s41396-020-00742-8
- Shukla, M.K., Watanabe, A., Wilson, S., Giovagnetti, V., Moustafa, E.I., Minagawa, J., and Ruban, V. A. (2020). A novel method produces native light-harvesting complex II aggregates from the photosynthetic membrane revealing their role in nonphotochemical quenching. *J. Biol. Chem.* 295, 17816–17826. DOI: 10.1074/jbc.RA120.016181
- 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
- Watanabe, A., and Minagawa, J. (2020). Structural characterization of the photosystems in the green alga *Chlorella sorokiniana*. *Planta* 252, 79. DOI: 10.1007/s00425-020-03487-y

## 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

Visiting Scientist: FURUTANI, Masahiko

Visiting Graduate Student: MORI, Shogo

Technical Assistant: HAMADA, Mayako

MIYOSHI, Kikumi

YAMADA, Yuka

SOMA, Yoriko

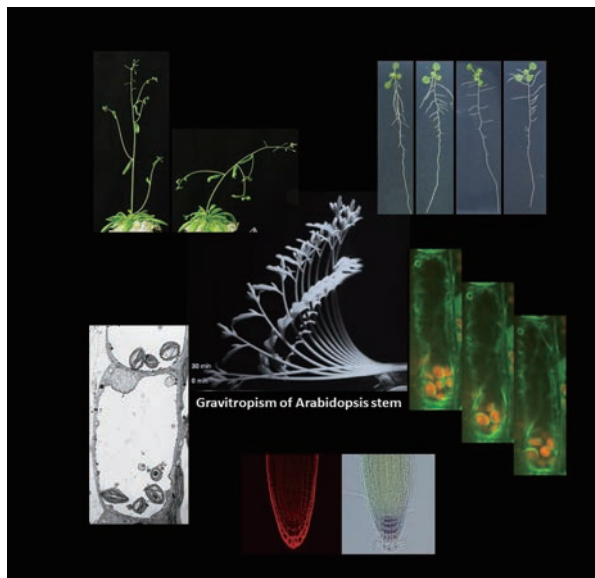
INOUE, Fumiko

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*)<sub>2</sub>, *LZY*<sub>3</sub>, and *LZY*<sub>4</sub> 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. At that time, we utilized fixed and cleared root samples because *LZY3*-mCherry is barely detected in live cell imaging; probably due to low expression levels and the tissue depth of its root cap. We subsequently generated *LZY4p:LZY4-mScarlet* transgenic lines which complemented the *lzy4* mutant. We then succeeded in live cell imaging of *LZY4*-mScarlet in the columella cells by using vertical-stage confocal microscope equipped with a high sensitivity EMCCD camera. *LZY4*-mScarlet was polarly localized on the basal side of the plasma membrane, in a similar way to that which *LZY3*-mCherry was localized. In addition, *LZY4*-mScarlet was found to be localized at the periphery of amyloplasts. We are now focusing on the dynamics of *LZY4*-mScarlet in response to gravistimulation and also on the mechanism of localization to the plasma membrane and amyloplast periphery.

### 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.

#### 2-1 Determination of GSA in roots

It has been reported that primary and lateral roots of *lzy2;3;4* triple mutants showed negative gravitropism. We refer to the phenotype of the reversed growth direction in the *lzy* triple mutant as "anti-gravitropic". It has been proposed that GSA is determined by balancing two opposing growth components: gravitropism and "anti-gravitropic" offsets (AGO). We assumed that the balance between gravitropism and AGO was disrupted in *lzy2;3;4* mutants, and hypothesized that AGO would be manifested as an "anti-gravitropic" phenotype. To investigate the mechanism of GSA control, we performed genetic analyses with *lzy2;3;4* mutants and *pgm* (Figure 1). Since *pgm* is a starch-less mutant, gravity sensing ability was greatly reduced in this type of mutant, resulting in varied growth direction (Figure 1D and H). The *pgm* mutation suppressed the "anti-gravitropic" phenotype of *lzy2;3;4* triple mutants (Figure 1B, C, F, and G). This finding indicates that gravitropic and "anti-gravitropic" growth is likely to share a similar gravity-sensing mechanism in primary roots.

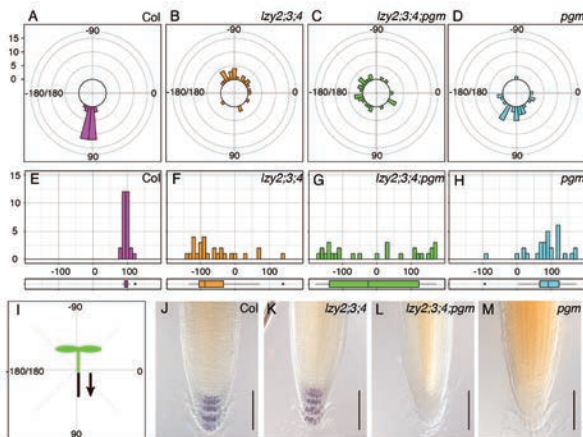


Figure 1. Starchless mutation affects “anti-gravitropic” response in roots. Polar distribution of the growth direction of primary root in wild-type Col (A), *lzy2;3;4* (B), *lzy2;3;4;pgm* (C), and *pgm* (D). Growth angles were plotted on histograms and distributions were evaluated with boxplots from wild-type Col (E), *lzy2;3;4* (F), *lzy2;3;4;pgm* (G), and *pgm* (H). Schematic representation of growth angle quantification (I). Each bar indicates the number of roots. Lugol’s staining of primary roots of wild-type Col (J), *lzy2;3;4* (K), *lzy2;3;4;pgm* (L), and *pgm* (M). Bars indicate 100  $\mu$ m.

### 2-2 Determination of GSA in shoots

The primary shoots of *lzy1;2;3* triple mutants exhibited non-responsiveness to gravistimulation, so it was expected that the lateral branches of the triple mutant would grow horizontally like those of *sgr* mutants. However, they unexpectedly grew downward and showed positive gravitropism upon gravistimulation (Figure 2). As amyloplast sedimentation is thought to be important for gravity sensing in shoot gravitropism as well as root gravitropism, we examined whether starch-accumulated amyloplasts are required for the “anti-gravitropic” phenotype of lateral branches in the *lzy1;2;3* mutants. Although no clear difference was observed in the growth angles of lateral branches by adding *pgm* mutation in *lzy1;2;3* mutants, the *pgm* mutation delayed the “anti-gravitropic” response in *lzy1;2;3* plants upon gravi-stimulation (Figure 2C, D and E).

We then investigated the relationship between the gravity sensing tissues for shoot gravitropism and the “anti-gravitropic” phenotype of lateral branches in *lzy1;2;3* plants. The endodermis is the gravity-sensing tissue in shoots, and *eall* is classified as an agravitropic mutant due to defect in its endodermis differentiation and because its lateral branches grow horizontally. The *eall* mutation clearly suppressed

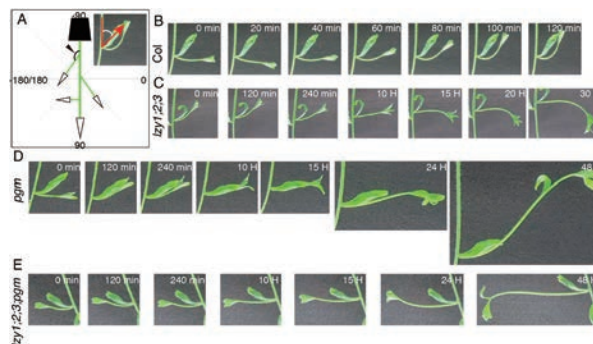


Figure 2. The gravitropic responses of lateral branches. A. Graphical image of the quantification of growth angles of lateral branches. Time-lapse images of lateral branches from wild-type Col (B), *lzy1;2;3* (C), *pgm* (D), *lzy1;2;3;pgm* (E) plants after inversion.

“anti-gravitropic” phenotype of *lzy1;2;3* triple mutants. In other words, the lateral branches of *eall lzy1;2;3* quadruple mutants grew horizontally. Overall, the endodermal tissue appears crucial for the normal gravitropism and “anti-gravitropic” phenotypes of lateral branches in *lzy1;2;3* triple mutants, but it remains unclear whether gravitropism and “anti-gravitropic” phenotypes share a similar gravity-sensing mechanism in shoots.

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

RLD proteins were isolated as interactors of LZY. RLD1 and RLD4 function in gravitropism of primary roots and the GSA control of lateral roots. We have also shown that *RLD1*, *RLD2*, *RLD3*, and *RLD4* redundantly function in plant morphogenesis, the morphological abnormality gradually becomes more severe by multiplying *rdl* mutations (Figure 3). Indeed, *rdl1;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. Defects in root gravitropism and morphogenesis of *rdl* multiple mutants are associated with defective auxin transport. 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 plant 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. While *LZY1*, *LZY2*, and *LZY3*, has been shown to play a key role in the gravity signaling process in shoot endodermis, the role of RLDs in shoot gravitropism remains unclear because evaluating shoot gravitropism in *rdl* multiple mutants is difficult due to their abnormal morphology and retarded growth.

To investigate the importance of the BRX domain for RLD function, we examined whether *RLD3p:RLD3 $\Delta$ BRX-mClover3* could complement the morphological phenotype of *rdl2;3;4*. Unexpectedly, *RLD3 $\Delta$ BRX-mClover3* recovered the morphological abnormality of *rdl2;3;4* (Figure 3). Interestingly, a wider GSA phenotype possessing lateral branches was observed in *RLD3p:RLD3 $\Delta$ BRX-mClover3/rdl2;3;4*. Both of these indicate that RLD3 lacking the BRX domain retains the function for morphogenesis but not for the GSA phenotype. Moreover, when RLD3 was expressed under the control of the endodermis-specific ADF9 promoter in *RLD3p:RLD3 $\Delta$ BRX-mClover3/rdl2;3;4*, the GSA phenotype was significantly restored, thus suggesting that RLD is involved in gravity signaling in the endodermal cells in shoot gravitropism in a manner similar to LZYS. Furthermore, these results suggest that RLD3 without BRX domain retain general functions in membrane trafficking required for development, and that LZYS regulates the function of RLD via the BRX domain to conduct gravity signaling in the gravity sensing cells.



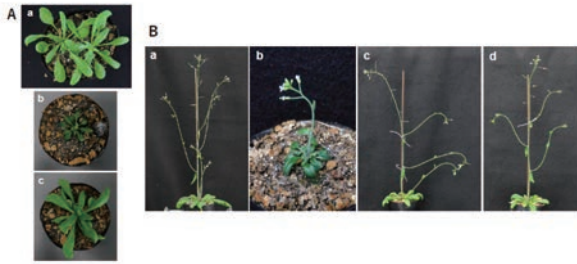


Figure 3. **A.** Phenotype of rosette leaves of wild-type, *rdl2;3;4* (b) and *RLD3p:RLD3ΔBRX-mClover3/rdl2;3;4*. **B.** The growth angle of shoot in wild-type, *rdl2;3;4* (b), *RLD3p:RLD3ΔBRX-mClover3/rdl2;3;4* (c), *ADF9p:RLD3/RLD3p:RLD3ΔBRX-mClover3/rdl2;3;4* (d).

#### IV. Re-orientation mechanism of the polarized growing root hair cell

Root hairs, thin tubular cells grown outward from the root surface, increase root surface area and the uptake of nutrients and water from soils. They are typical polarized growing cells and grow straight and long in a cell-autonomous manner when they are grown in open air or within a liquid medium (Figure 4A). However soils are filled with soil particles like grains of sand of various sizes or silt, which may interfere with the growth of root hairs (Figure 4B). We have developed an imaging system based on microfluidics to observe how root hairs grow in soils, since soils are not transparent enough to conduct observations within them through the use of microscopes (Figure 4C-E). Applying this system, paths or objects (2~250  $\mu\text{m}$ ) are formed in a transparent silicone-rubber to mimic the condition of soil particles (Figure 4D, E). It enables us to visualize Arabidopsis roots including root hairs that come into contact with obstacles in the microfluidic device. We found that root hairs grow along the surface of the obstacles, which supports the role of root hairs on uptake of nutrients and water from soil particles (Figure 4E). To further get insight into the mechanism of root hair growth in soils, we have been analyzing various regulators that are important for normal root hair growth with the imaging system. We found that two protein kinases are regulators for re-orientation of root hair growth.

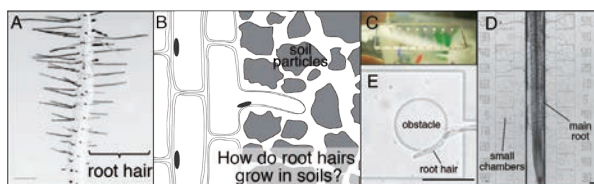


Figure 4. Live-imaging of root hairs. **A.** An Arabidopsis root grown on the Agar medium. **B.** Illustration of root hairs in soils. **C.** A micro-fluidic device for imaging of Arabidopsis root hairs. **D.** Main roots grow in a main path within the device, and root hairs elongate into small chambers aligned beside the main path. **E.** A root hair elongating along an obstacle set in a small chamber. Bars indicate 200  $\mu\text{m}$  or 100  $\mu\text{m}$  in (A) or (D, E), respectively.

#### Publication List:

##### [Original papers]

- Abe, Y., Meguriya, K., Matsuzaki, T., Sugiyama, T., Yoshikawa, H.Y., Morita, M.T., and Toyota, M. (2020). Micromanipulation of amyloplasts with optical tweezers in *Arabidopsis* stems. *Plant Biotechnol.* 37, 405–415. DOI: 10.5511/plantbiotechnology.20.1201a
- Kawamoto, N., Kanbe, Y., Nakamura, M., Mori A., Terao Morita, M.T. (2020). Gravity-sensing tissues for gravitropism are required for “anti-gravitropic” phenotypes of *lzy* multiple mutants in *Arabidopsis*. *Plants* 9. DOI: 10.3390/plants9050615
- Shindo, M., Makigawa, S., Kodama, K., Sugiyama, H., Matsumoto, K., Iwata, T., Wasano, N., Kano, A., Morita, M.T., and Fujii, Y. (2020). Design and chemical synthesis of root gravitropism inhibitors: Bridged analogues of ku-76 have more potent activity. *Phytochemistry* 179. DOI: 10.1016/j.phytochem.2020.112508
- Shindo, M., Makigawa, S., Matsumoto, K., Iwata, T., Wasano, N., Kano, A., Morita, M.T., and Fujii, Y. (2020). Essential structural features of (2Z,4E)-5-phenylpenta-2,4-dienoic acid for inhibition of root gravitropism. *Phytochemistry* 172. DOI: 10.1016/j.phytochem.2020.112287
- Tsugawa, S., Sano, T.G., Shima, H., Morita, M.T., and Demura, T. (2020). A mathematical model explores the contributions of bending and stretching forces to shoot gravitropism in *Arabidopsis*. *Quant. Plant Biol.* 1, e4. DOI: 10.1017/qpb.2020.5

## LABORATORY OF GENOME INFORMATICS

Associate Professor  
UCHIYAMA, Ikuro

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 groups on the fly by using a specified set of organisms. MBGD also has pre-calculated ortholog tables for each major taxonomic group, and provides several viewing modes to display the entirety of each ortholog table. For some closely related taxa, MBGD provides conserved synteny information calculated using the CoreAligner program. MBGD additionally provides a ‘MyMBGD’ mode, which allows users to add their own genomes to MBGD. MBGD now contains 6318 genomes, including 5861 bacteria, 254 archaea, and 203 eukaryota. These data sets are classified based on the hierarchical ortholog classification strategy described in the section below.

We continue to develop the database and the application software utilizing the database to enhance its usability. As an application software for analyzing novel genome sequences based on the database, we are developing 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 Genomagle software to calculate the module completion ratio for each KEGG Module

entry (in collaboration with Dr. Takami, Univ Tokyo) and developed a novel interface to compare the presence or absence of functional modules among specified organisms (Figure 1).



Figure 1. Comparative display of KEGG module completion among different genomes based on the MBGD-Genomagle combined analysis.

### 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 now used to construct the standard ortholog table covering all the representative genomes stored in MGD.

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

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

We have developed a comparative genomics workbench named RECOG (Research Environment for COmparative Genomics), which aims to extend the current MGD 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 struc-

ture of the ortholog information in the Semantic Web, we developed an ortholog ontology (OrthO) and described the ortholog information in MGD in the form of the Resource Description Framework (RDF). OrthO is now superseded by the Orthology Ontology (ORTH) that integrates OrthO and other ontologies and is adopted by other orthology resources.

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

#### VI. A novel approach for identification of genomic islands

Genomes of bacterial species can show great variation in their gene content, and thus systematic analysis of the entire gene repertoire, termed the “pan-genome”, is important for understanding bacterial intra-species diversity. As we have already developed a procedure (CoreAligner) to define the core genome as the genes conserved among the genomes of the given species, characterizing the remaining part of the genomes (non-core genomes) should be important for understanding the species’ diversity. For this purpose, we developed a method (FindMobile) to define mobility of genes against the reference coordinate determined by the core genome alignment. We are now 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 MGD 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]

- Iida, M., Suzuki, M., Sakane, Y., Nishide, H., Uchiyama, I., Yamamoto, T., Suzuki, K.T., and Fujii, S. (2020). A simple and practical workflow for genotyping of CRISPR-Cas9-based knockout phenotypes using multiplexed amplicon sequencing. *Genes to Cells* 25, 498–509. DOI: 10.1111/gtc.12775
- Nagao, R., Kato, K., Ifuku, K., Suzuki, T., Kumazawa, M., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., *et al.* (2020). Structural basis for assembly and function of a diatom photosystem I-light-harvesting supercomplex. *Nat. Commun.* 11. DOI: 10.1038/s41467-020-16324-3
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**LABORATORY FOR SPATIOTEMPORAL REGULATIONS**



Associate Professor  
**NONAKA, Shigenori**

*Specially Appointed Assistant Professor :*

*YOKE, Hiroshi*

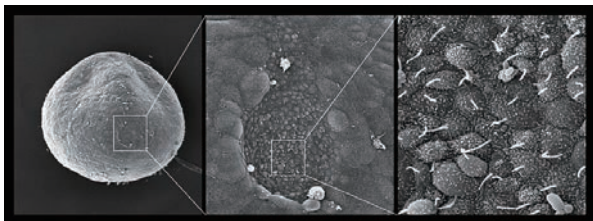
*Postdoctoral Fellow: TANIGUCHI, Atsushi*

*Visiting Scientist: YANASE, Ryuji*

*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.

We pursued this question by measuring  $Ca^{2+}$  dynamics in

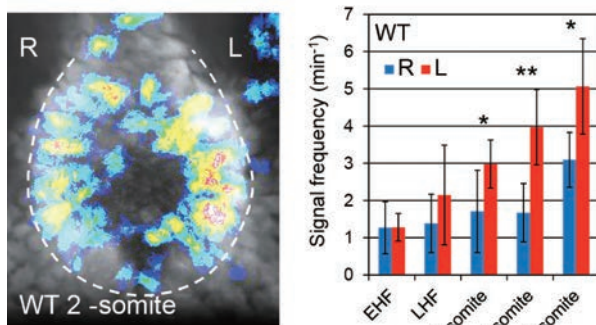


Figure 1. Left: Distribution of  $Ca^{2+}$  elevation in a 2-somite wild-type node. Right: Time course of  $Ca^{2+}$  elevation frequency at the left and the right sides.

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<sup>-/-</sup>* 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 ezDSLMS, 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.

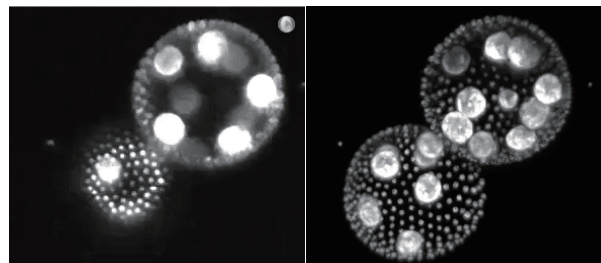


Figure 2. Images of floating volvox taken by ezDSLMS with ETL. Left: Single optical section. Right: Maximum intensity projection.

**Publication List:**

[Original papers]

- Hattori, Y., Naito, Y., Tsugawa, Y., Nonaka, S., Wake, H., Nagasawa, T., Kawaguchi, A., and Miyata, T. (2020). Transient microglial absence assists postmigratory cortical neurons in proper differentiation. *Nat. Commun. 11*. DOI: 10.1038/s41467-020-15409-3
- Kondow, A., Ohnuma, K., Kamei, Y., Taniguchi, A., Bise, R., Sato, Y., Yamaguchi, H., Nonaka, S. and Hashimoto, K. (2020). Light-sheet microscopy-based 3D single-cell tracking reveals a correlation between cell cycle and the start of endoderm cell internalization in early zebrafish development. *Dev. Growth Differ. 62*, 495-502. DOI: 10.1111/dgd.12695

LABORATORY FOR BIOTHERMOLOGY



Specially Appointed Associate Professor  
KAMEI, Yasuhiro

- Specially Appointed Assistant Professor :  
SAKAMOTO, Joe  
Postdoctoral Fellow: KAMIKAWA, Yuko  
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

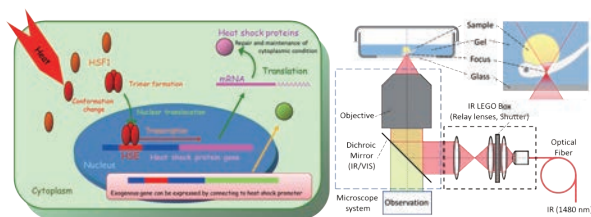


Figure 1. Schematic illustration of heat shock response in cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

to analyze the thermodynamics of a cell via the use of a nano-scale thermometer; the second of our abovementioned original techniques. To analyze temperature distribution at subcellular resolutions, a nano-scale thermometer is required. For this reason, we have focused on fluorescent proteins, and developed a genetically encoded thermometer, the so called gTEMP, with Dr. Takeharu Nagai from Osaka University (Nakano *et al*, PLoS One 2017). This thermometer has some unique properties, such as rapid 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.* *Arabidopsis* 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).

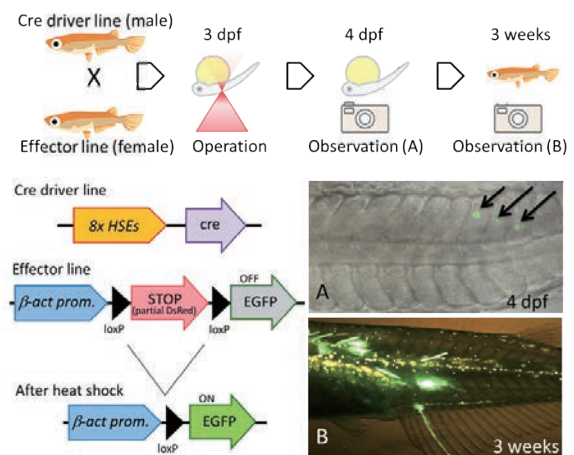


Figure 2. Long-term gene expression using the cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

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]

- Ashida, Y., Honma, Y., Miura, N., Shibuya, T., Kikuchi, H., Tamada, Y., Kamei, Y., Matsuda, A. and Hattori, M. (2020). Imaging performance of microscopy adaptive-optics system using scene-based wavefront sensing. *J. Biomed. Optics*-25, 123707. DOI: 10.1117/1.jbo.25.12.123707
- Kanazawa, N., Goto, M., Harada, Y., Takimoto, C., Sasaki, Y., Uchikawa, T., Kamei, Y., Matsuo, M. and Fukamachi, S. (2020). Changes in a cone opsin repertoire affect color-dependent social behavior in medaka but not behavioral photosensitivity. *Front. Genet. 11*. DOI: 10.3389/fgene.2020.00801
- Kondow, A., Ohnuma, K., Kamei, Y., Taniguchi, A., Bise, R., Sato, Y., Yamaguchi, H., Nonaka, S. and Hashimoto, K. (2020). Light-sheet microscopy-based 3D single-cell tracking reveals a correlation between cell cycle and the start of endoderm cell internalization in early zebrafish development. *Dev. Growth Differ. 62*, 495-502. DOI: 10.1111/dgd.12695
- Nishiike, Y., Miyazoe, D., Togawa, R., Yokoyama K., Nakasone, K., Miyata, M., Kikuchi, Y., Kamei, Y., Todo, T., Ishikawa-Fujiwara, T., Ohno, K., *et al.* (2021). Estrogen receptor 2b is the major determinant of sex-typical mating behavior and sexual preference in medaka. *Curr. Biol. 31*, 1699+. DOI: 10.1016/j.cub.2021.01.089
- Watanabe, Y., Okuya, K., Takada, Y., Kinoshita M., Yokoi, S., Chisada, S., Kamei, Y., Tatsukawa, H., Yamamoto, N., Abe, H., Hashimoto, H., *et al.* (2020). Gene disruption of medaka (*Oryzias latipes*) orthologue for mammalian tissue-type transglutaminase (TG2) causes movement

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### [Review article]

- Abe, G., Hayashi, T., Yoshida, K., Yoshida, T., Kudoh, H., Sakamoto, J., Konishi, A., Kamei, Y., Takeuchi, T., Tamura, K., *et al.* (2020). Insights regarding skin regeneration in non-amniote vertebrates: Skin regeneration without scar formation and potential step-up to a higher level of regeneration. *Semin. Cell Dev. Biol. 100*, 109–121. DOI: 10.1016/j.semcdb.2019.11.014



## 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

**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 2020, we 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 17 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.

During 2019, we carried out 57 NGS projects in collaboration with researchers from academic institutions throughout the world. These projects cover a wide range of species (bacteria, animals, plants, and fungi) including both model and non-model organisms, and various other applications such as genomic re-sequencing, RNA-seq and CHIP-seq.



Figure 1. Next-generation sequencer

### Proteomics

As is listed below, two types of mass spectrometers and two protein sequencers are used for proteome studies in our facility. In 2019, we analyzed approximately 1000 samples with mass spectrometers and protein sequencers.

- LC-MS (AB SCIEX TripleTOF 5600 system)
- LC-MS (Thermo Fisher SCIENTIFIC Orbitrap 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 (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500)
- Ultracentrifuge (Beckman XL-80XP etc.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1)

### Publication List on Cooperation:

#### [Original Papers]

- Araki, K.S., Nagano, A.J., Nakano, R.T., Kitazume, T., Yamaguchi, K., Hara-Nishimura, I., Shigenobu, S., and Kudoh, H. (2020). Characterization of rhizome transcriptome and identification of a rhizomatous ER body in the clonal plant *Cardamine leucantha*. *Sci. Rep.* 10. DOI: 10.1038/s41598-020-69941-9
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- Fukutomi, Y., Kondo, S., Toyoda, A., Shigenobu, S., and Koshikawa, S. (2021). Transcriptome analysis reveals wingless regulates neural development and signaling genes in the region of wing pigmentation of a polka-dotted fruit fly. *FEBS J.* 288, 99–110. DOI: 10.1111/febs.15338
- Gu, N., Tamada, Y., Imai, A., Palfalvi, G., Kabeya, Y., Shigenobu, S., Ishikawa, M., Angelis, K.J., Chen, C., and Hasebe, M. (2020). DNA damage triggers reprogramming of differentiated cells into stem cells in *Physcomitrella*. *Nat. Plants* 6, 1098+. DOI: 10.1038/s41477-020-0745-9
- Li, Y., Omori, A., Flores, R.L., Satterfield, S., Nguyen, C., Ota, T., Tsurugaya, T., Ikuta, T., Ikeo, K., Kikuchi, M., *et al.* (2020). Genomic insights of body plan transitions from bilateral to pentameral symmetry in Echinoderms. *Commun. Biol.* 3. DOI: 10.1038/s42003-020-1091-1
- Mondal, S.I., Akter, A., Koga, R., Hosokawa, T., Dayi, M., Murase, K., Tanaka, R., Shigenobu, S., Fukatsu, T., and Kikuchi, T. (2020). Reduced genome of the gut symbiotic bacterium “*Candidatus Benitsuchiphilus tojoi*” provides insight into its possible roles in ecology and adaptation of the host insect. *Front. Microbiol.* 11. DOI: 10.3389/fmicb.2020.00840
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#### [Research activity by Shuji Shigenobu]

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

## SPECTROGRAPHY AND BIOIMAGING FACILITY



Specially Appointed Associate Professor  
KAMEI, Yasuhiro

Technical Staff: KONDO, Maki  
TANIGUCHI-SAIDA, Misako  
Technical Assistant: ICHIKAWA, Chiaki  
ASAO, Momoko  
NAKAGAWA, Mami



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.

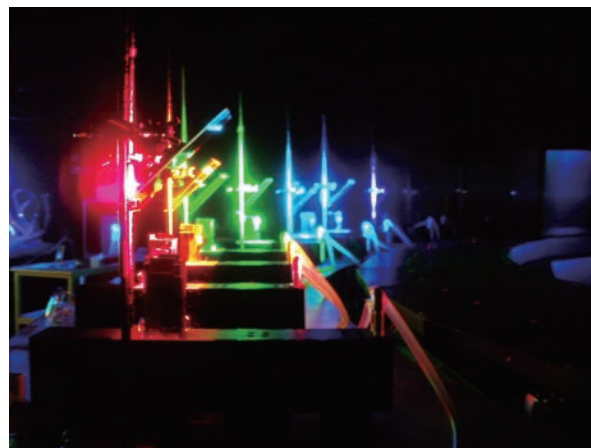


Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously onto samples stored in cooling chambers.

### Microscopes

This facility also provides bioimaging machinery, 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.

The light-sheet microscope was developed by Dr. Ernst Stelzer's group at the European Molecular Biology

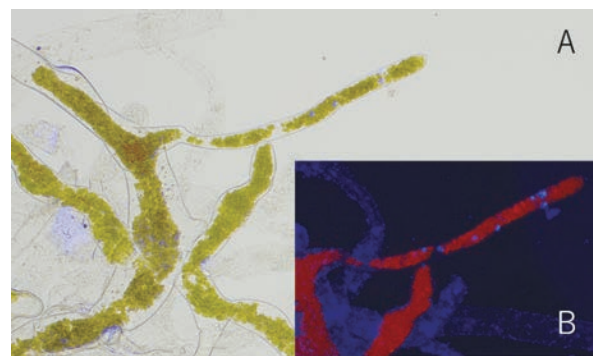


Figure 2. Microscopic images of green algae, *Codium fragile*. This sample was stained by hoechst33342. A) Bright and blue fluorescent merge image. B) Blue fluorescent and red fluorescent merge image. Images were taken by a visitor from a cooperation program with Osaka City University (Sekii, Soichiro).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



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 (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 2020, we held the 8<sup>th</sup> 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 (Figure 3) for the first time due to the COVID-19 pandemic.

We have also held annual "Bioimaging Forum" events, which discuss bioimaging from various technical perspectives such as that of microscopy, new photo-technology, and computer science. This year we planned on staging the 14<sup>th</sup> edition of this event in November with a focus on micro CT technology. Like so many events of its type, this meeting was also to be held in an online format due to the global COVID-19 pandemic.



Figure 3. A scene from the 8th Bio-Imaging Analysis Training Course. The course was held in an online meeting format to prevent COVID-19 infection. The 40 participants were able to interact with lecturers via the use of Zoom and chat applications from their specific locations.

### Publication List on Cooperation

#### [Original papers (Selected)]

- Ashida, Y., Honma, Y., Miura, N., Shibuya, T., Kikuchi, H., Tamada, Y., Kamei, Y., Matsuda, A., Hattori, M. (2020) Imaging performance of microscopy adaptive-optics system using scene-based wavefront sensing. *J. Biomed. Optics*, 25, 123707.
- Kanazawa, N., Goto, M., Harada, Y., Takimoto, C., Sasaki, Y., Uchikawa, T., Kamei, Y., Matsuo, M. and Fukamachi, S. (2020) Changes in a cone opsin repertoire affect color-dependent social behavior in Medaka but not behavioral photosensitivity. *Front. Genetics* 11, Article 801.
- Kawamoto, N., Carpio, D. P., Hofmann, A., Mizuta, Y., Kurihara, D.,

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- Kondow, A., Ohuma, K., Kamei, Y., Taniguchi, A., Bise, R., Sato, Y., Yamaguchi, H., Nonaka, S., Hashimoto, K. (2020) Light-sheet microscopy-based 3D single-cell tracking reveals a correlation between cell cycle and the start of endoderm cell internalization in early zebrafish development. *Dev. Growth Differ.* 62, 495-502.
- Sakai, Y., Kato, K., Koyama, H., Kuba, A., Takahashi, H., Fujimori, T., Hatta, M., Negri, A. P., Baird, A. H. and Ueno, N. (2020) A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, *Acropora tenuis*. *Sci Rep.* 10, 17680.
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- Yokoi, S., Naruse, K., Kamei, Y., Ansai, S., Kinoshita, M., Mito, M., Iwasaki, S., Inoue, S., Okuyama, T., Nakagawa, S., Young, L. J., Takeuchi, H. (2020) Sexually dimorphic role of oxytocin in medaka mate choice. *PNAS*, 117, 4802-4808.

#### [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

Technical Assistant: SUGIURA, Hiroki  
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



Figure 1. Biological Information Analysis System

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 (*Pleurodeles waltl*), The Plant Organelles Database, MBGD (microbial genomes), and DB-HABs (harmful algal blooms).

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

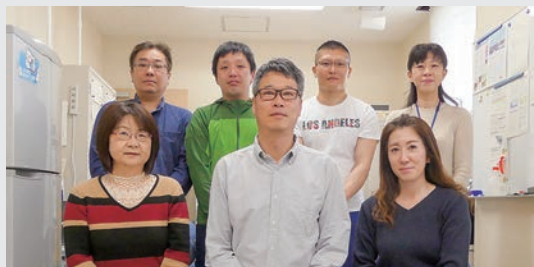


Head  
FUJIMORI, Toshihiko

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<sup>2</sup> 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, 2020 to March 31, 2021, 2,813 mice (2 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 34,445 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 (8 transgenic lines), and stored using cryopreservation (46 transgenic lines). The frozen eggs of 132 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



Figure 4. Liquid nitrogen tank

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.

In 2020, 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.



Figure 5. Vinyl isolators in the quarantine room

## 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

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



The Model Plant research facility manages facilities for the cultivation of plants in general, and the rearing of several animal species that do not qualify to be housed in other facilities.

This facility equips and manages around 75 culture boxes or growth chambers, as well as 13 rooms with the P1P

physical containment level required for established and emerging model plants, such as the thale cress *Arabidopsis thaliana*, the rice *Oryza sativa*, the moss *Physcomitrella patens*, the liverwort *Marchantia polymorpha*, the green alga *Chlamydomonas reinhardtii* and several other flowering plants which include several carnivorous plants. The facilities are also used to grow the sea anemone *Exaiptasia pallida*. Most culture space is used throughout the year by more than 70 researchers from both external and internal groups.

As well as regular culture conditions, extreme environmental conditions for light and temperature are available for various types of experiments. Three chambers (3.4 m<sup>2</sup> each) that can control CO<sub>2</sub> and humidity in addition to temperature and light (max 70,000 lux) conditions are also available. Furthermore, a tissue culture rack with dimming LEDs and pulse-width modulation controllers are used for algae cultures which are exposed to precise light concentrations. Autotrophic and heterotrophic culture devices are also available for researchers using cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches and a safety cabinet. In addition, several analytical instruments, that include two flow cytometry systems and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available. On top of this, a liquid handling system for fully automated in situ hybridization of sections of up to 60 glass slides simultaneously is also provided.

A 386-m<sup>2</sup> experimental farm next to the NIBB Myodaiji area building is maintained for Japanese morning glories and related *Ipomoea* species, several carnivorous plants, the castor bean, and other flowering plants that must be cultivated outdoors. Three heated greenhouses (44, 44, and 45 m<sup>2</sup>) are used for the sensitive carnivorous plants and a periodical mass-flowering plant, *Strobilanthes flexicaulis*. Four air-conditioned greenhouses (4, 6, 9, and 9 m<sup>2</sup>) are provided for the cultivation of Japanese morning glories and several carnivorous plants. Two air-conditioned greenhouses (9 and 18 m<sup>2</sup>) meet the P1P physical containment level and are available for experiments using transgenic rice plants, Japanese morning glories, as well as carnivorous plants. The Plant Culture Laboratory also maintains a 46 m<sup>2</sup> building with a storage area and workspace. Part of this building is used for the rearing of Japanese rhinoceros beetles and the common grass yellow butterfly.



Figure 6. Webcam monitoring of Japanese rhinoceros beetle larvae.

Between April 2020 and March 2021, 4 culture boxes were replaced by newer ones.

### CELL BIOLOGY RESEARCH FACILITY

Associate Professor: WATANABE, Eiji

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



Figure 7. 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.

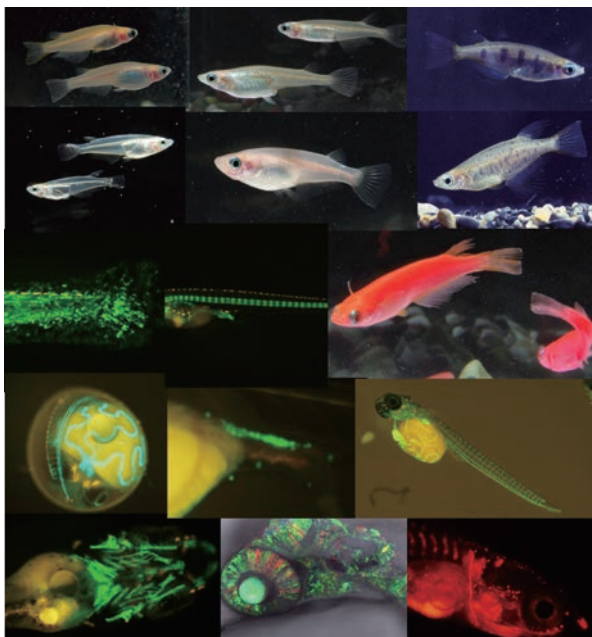
### I. NBRP Medaka (*Oryzias latipes*)

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 illumina-



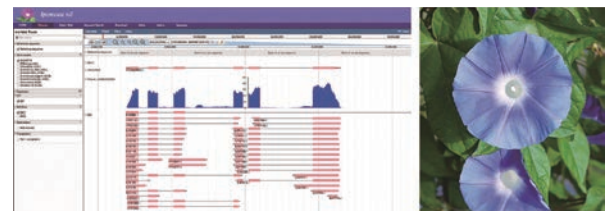
Medaka resources provided from NBRP medaka

tion 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 2020, All on-site conferences were cancelled due to the new coronavirus pandemic, and public relations activities were shifted to online. As a new attempt under this situation, NIBB and the ICOB, Academia Sinica, Taiwan, jointly organized the “NIBB-Academia Sinica International Webinar of Aquatic Model Organisms for Basic Biology to Human Disease Models” Total of 100 peoples from Japan, Taiwan, China, Indonesia, India, Singapore, Germany, Poland, Switzerland, the Netherlands, Ukraine, the U.S., Canada, and Argentina registered for the webinar. The active use of webinars, which are not limited by location, is one way to promote NBRP medaka under the new coronavirus era.

### II. NBRP Morning Glory (*Ipomoea nil*)

Project Manager: HOSHINO, Atsushi

The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, that is studied worldwide, and is especially 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, and collaborates with the core organization center at Kyushu University. From April 2020 to March 2021, we have also collected several mutant lines, and provided 14 mutant lines and 39 DNA clones to both local and international biologists. We also analyzed the whole genome sequences of the 100 mutant lines using next-generation sequencers to develop databases for genetic variations.



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

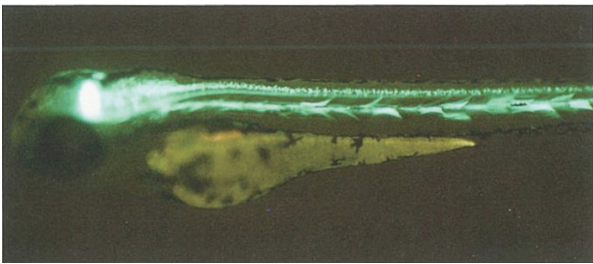
### III. NBRP Zebrafish (*Danio rerio*)

Project Manager: HIGASHIJIMA, Shin-ichi

NIBB is a sub-center of the National BioResource Project (NBRP) Zebrafish, and collaborates with the core organization center, RIKEN Brain Science Institute. We mainly collect zebrafish strains expressing fluorescent proteins in specific cells of the central nervous system and distribute them to researchers worldwide. The zebrafish is an important and globally used experimental vertebrate model animal with a simple body structure. It can be genetically manipulated, and its embryos are transparent enough for optical obser-



vation. Research using zebrafish for the studies of neural development and neural circuit functions are growing rapidly worldwide, and the importance of strains collected and provided by NIBB to researchers is growing accordingly.



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

## NIBB CENTER FOR THE INTERUNIVERSITY BIO-BACKUP PROJECT (IBBP CENTER)



Head and Specially Appointed Professor  
NARUSE, Kiyoshi

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



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

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



Figure 1. IBBP Center.

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.

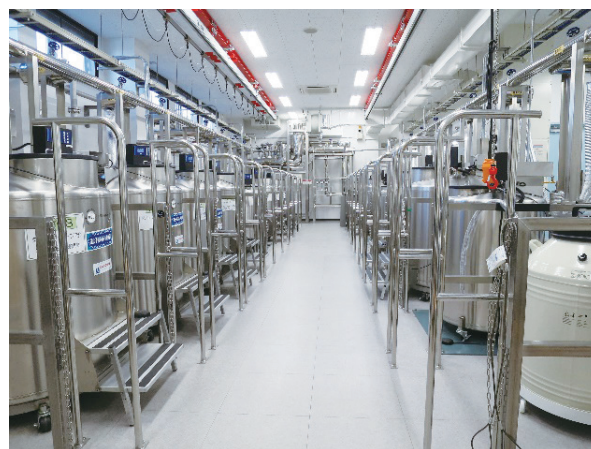


Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

### I. Current status of back up available for biological resources

In 2020, the IBBP Center stored 5,264 384-well and 112 96-well plates consisting of 2,244,864 clones as cDNA/BAC clones, 20,163 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 5,114 133mm-straw tubes for sperm and 664 seed samples. In total 2,058,069 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 2020, we have conducted 10 collaborative research projects aimed at achieving these goals. We also worked to establish a research center for cryo-biological study through this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2020 on November 26-27, 2020 online, because of the COVID-19 pandemic. We had 195 participants from several fields covering physics, chemistry, biology, and technology. In the special lecture, there was a presentation on the effect of polymeric compounds on cell cryopreservation and its mechanism of action, and other presentations related to the development of cryopreservation technology, such as methods for preserving insects, plants, and animal embryos.



Figure 4. Group photo of Cryopreservation conference 2020

### Publication List on Cooperation:

#### [Original papers]

- Watanabe, H., and Akiyama, Y. (2020). Improved and reproducible cell viability in the superflash freezing method using an automatic thawing apparatus. *Cryobiology*, 96, 12-18. DOI:10.1016/j.cryobiol.2020.09.003
- Kawasaki, Y., Kohaya, N., Shibao, Y., Suyama, A., Kageyama, A., Fujiwara, K., Kamoshita, M., Matsumura, K., Hyon, S-H., Ito J. and Kashiwazaki, N. (2020). Carboxylated  $\epsilon$ -poly-L-lysine, a cryoprotective agent, is an effective partner of ethylene glycol for the vitrification of embryos at various preimplantation stages. *Cryobiology*, 97, 245-249. DOI:10.1016/j.cryobiol.2020.10.004



## CENTER FOR THE DEVELOPMENT OF NEW MODEL ORGANISMS



Head  
UENO, Naoto



Professor  
SHIGENOBU, Shuji



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

Professor: MINAGAWA, Jun  
KAWAGUCHI, Masayoshi  
NIIMI, Teruyuki  
Assistant Professor: HOSHINO, Atsushi  
Postdoctoral Fellow: KISHIMOTO, Mariko  
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

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 CRISPR-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* cris pant in *P. waltl*. A knock-out founder of tyrosinase, a melanin synthesis enzyme, and wild newt (left and right, respectively). *tyr* cris pant 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.

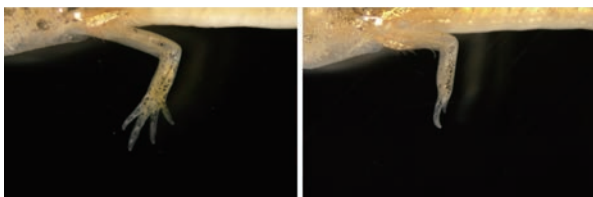


Figure 2. A limb-specific enhancer (ZRS/MFCS1) of *sonic hedgehog* crispant in *P. waltl*. Phenotypes of limb regeneration in wild and ZRS/MFCS1 crispant (left and right, respectively). Unlike in normal limb regeneration in the wild type, severe reduction of digit formation was seen in ZRS/MFCS1 crispant.

### Publication List:

#### [Original papers]

- Habuta, M., Yasue, A., Suzuki, K.T., Fujita, H., Sato, K., Kono, H., Takayama, A., Bando, T., Miyaishi, S., Oyadomari, S., *et al.* (2020). Fgf10-CRISPR mosaic mutants demonstrate the gene dose-related loss of the accessory lobe and decrease in the number of alveolar type 2 epithelial cells in mouse lung. *PLoS One* 15. DOI: 10.1371/journal.pone.0240333
- Iida, M., Suzuki, M., Sakane, Y., Nishide, H., Uchiyama, I., Yamamoto, T., Suzuki, K.T., and Fujii, S. (2020). A simple and practical workflow for genotyping of CRISPR-Cas9-based knockout phenotypes using multiplexed amplicon sequencing. *Genes to Cells* 25, 498–509. DOI: 10.1111/gtc.12775
- Sanoh, S., Hanada, H., Kashiwagi, K., Mori Tsukasa and Goto-Inoue, N., Suzuki, K.T., Mori, J., Nakamura, N., Yamamoto, T., Kitamura, S., Kotake, Y., *et al.* (2020). Amiodarone bioconcentration and suppression of metamorphosis in *Xenopus*. *Aquat. Toxicol.* 228. DOI: 10.1016/j.aquatox.2020.105623

## CENTER FOR RADIOISOTOPE FACILITIES



Head  
HASEBE, Mitsuyasu



Associate Professor  
KODAMA, Ryuji

**Technical Staff:**

**MATSUDA, Yoshimi**  
(Radiation Protection Supervisor, Myodaiji area)  
**SAWADA, Kaoru**  
(Radiation Protection Supervisor, Yamate area)  
**IINUMA, Hideko**  
**HAYASHI, Tomoko**

**Technical Assistant:**



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 (Table 1) and monitor the purchase and transfer of radioisotopes. This year, the staff also worked to close down the CRF Yamate branch (Figure 1, Tables 2-4).

With the emergence of non-radioisotope alternative technologies, the number of CRF users has gradually declined over the past 20 years (Figure 1). To improve efficiency, the director general of the NIBB proposed concentrating the CRF to the Myodaiji branch and closing the Yamate branch. This possibility was discussed by the CRF Steering Committee; it was decided during the Directors' Meeting of the three Okazaki Institutes (NIBB, NIPS, and IMS) in September 2020 that the Yamate branch would be closed and the CRF would be consolidated to the Myodaiji branch. The CRF center staff confirmed that there was no contamination at the Yamate branch and transferred radioisotopes to the Myodaiji branch for further use or to the Japan Radioisotope Association for proper disposal. In March 2021, the Yamate branch was successfully decommissioned, and the CRF was centralized to the Myodaiji branch (Figure 2).

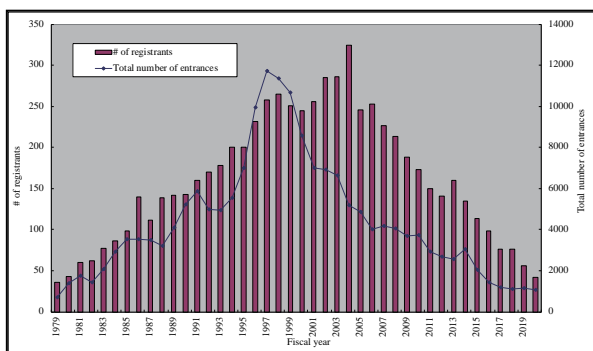


Figure 1. Annual number of registrants and total number of entrances to the CRF

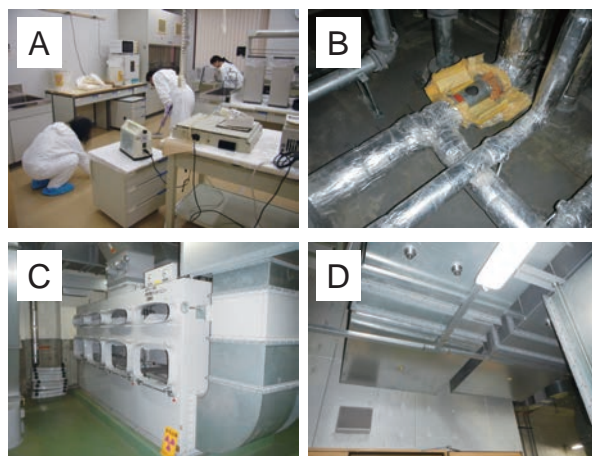


Figure 2. Absence of radiation contamination at the Yamate branch. Inspection of the floor (A), drainage pipes (B), filter units (C), and exhaust ducts (D).

Table 1. Training courses offered for radiation workers during fiscal year 2020.

| Training course                   | Branch   | # of participants |
|-----------------------------------|----------|-------------------|
| Introductory course for beginners | Myodaiji | 5                 |
| Introductory course for experts   | Myodaiji | 1                 |
| User training course              | Myodaiji | 28                |
| User training course              | Yamate   | 9                 |

Table 2. Number of registrants and users of the CRF during fiscal year 2020.

|                  | Myodaiji-branch | Yamate-branch |
|------------------|-----------------|---------------|
| # of registrants | 33              | 9             |
| # of users       | 14              | 6             |

Table 3. Total number of uses by individual users and visitors during fiscal year 2020.

|               | Myodaiji branch | Yamate branch |
|---------------|-----------------|---------------|
| # of users    | 631             | 265           |
| # of visitors | 119             | 44            |

Table 4. Radioisotopes received and used (in kBq) at each branch during fiscal year 2020.

|                  |          | Myodaiji branch | Yamate branch | Total   |
|------------------|----------|-----------------|---------------|---------|
| <sup>125</sup> I | Received | 0               | 0             | 0       |
| <sup>125</sup> I | Used     | 0               | 0             | 0       |
| <sup>35</sup> S  | Received | 0               | 0             | 0       |
| <sup>35</sup> S  | Used     | 0               | 0             | 0       |
| <sup>32</sup> P  | Received | 37,000          | 12,950        | 49,950  |
| <sup>32</sup> P  | Used     | 22,274          | 12,950        | 35,224  |
| <sup>14</sup> C  | Received | 3,913           | 0             | 3,913   |
| <sup>14</sup> C  | Used     | 15              | 0             | 15      |
| <sup>3</sup> H   | Received | 612,350         | 0             | 612,350 |
| <sup>3</sup> H   | Used     | 0               | 0             | 0       |



## 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<br>KAWAGUCHI, Masayoshi |
| Associate Professor: | MANO, Shoji<br>KODAMA, Ryuji              |
| Assistant Professor: | JOZUKA, Katsuki<br>FUJITA, Hironori       |

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

#### The group's main activities in 2020 were as follows:

- 1) Prepared reports on 2019 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 FY2020 third supplementary budget and FY2021 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 2019 NIBB activities for the annual external reviewer meeting on 29 January 2020, managed the meeting, and published the report ([https://www.nibb.ac.jp/pressroom/pdf/NIBBgaibu\\_19.pdf](https://www.nibb.ac.jp/pressroom/pdf/NIBBgaibu_19.pdf)).

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

8) Published the Annual Report 2019.

### PUBLIC RELATIONS GROUP

|  |  |
|--|--|
| Professor:                                     | FUJIMORI, Toshihiko                          |
| Specially Appointed Assistant Professor (URA): | KURATA, Tomoko                               |
| Technical Assistants:                          | BAN, Misato<br>HOSHINO, Maki<br>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 2020 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

We 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

This 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

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

##### 6) Organized scientific outreach programs

We organized online outreach events for the public and coordinated science classes for middle and high school students.

## LIAISON AND COOPERATION GROUP

|  |  |
|--|--|
| Professor:                                     | UENO, Naoto<br>HASEBE, Mitsuyasu<br>NARUSE, Kiyoshi<br>MORITA, Miyo T. |
| Specially Appointed Assistant Professor (URA): | TATEMATSU, Kiyoshi   |
| Technical Staff:                               | AKIMOTO-KATO, Ai   |
| Research Staff:                                | TANAKA, Ayako  |
| Technical Assistant:                           | TAKAHASHI, Ritsue<br>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 in 2020 were as follows:

#### 1) Coordinated the following conferences/symposia:

- Cryopreservation Conference 2020. Zoom online, November 26–27, 2020 (p. 102)
- NIBB-Academia Sinica International Webinar of Aquatic Model Organisms for Basic Biology to Human Disease Models. Zoom online, March 5, 2021 (p. 110-111)
- The Kick Off (1st) meeting for NIBB-COS Heidelberg International Collaborations Lecture series #1 "Plant Root Development". Zoom online, March 30, 2021 (p. 110)

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

- One young researcher from the Netherlands to be appointed as a specially appointed assistant professor (November 2020)
- One young researcher from Germany to conduct collaborative research with the Division of Environmental Photobiology (January 2021)

#### 3) Supported the following education-related programs:

- NIBB Internship Program 2020 (p. 116)

## COLLABORATIVE RESEARCH GROUP

|  |                                      |
|--|--------------------------------------|
| Professor:                                     | TAKADA, Shinji<br>SHIGENOBU, Shuji   |
| Specially Appointed Associate Professor (URA): | KAMEI, Yasuhiro                      |
| Associate Professor:                           | UCHIYAMA, Ikuo                       |
| Technical Assistant:                           | ICHIKAWA, Mariko<br>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 2020 were as follows:

- 1) Hosted 142 collaborative projects.
- 2) Operated and managed NOUS (NINS Operation Use System), an online platform to manage collaborative projects.
- 3) Developed remote and automated systems to support collaborative research during the COVID-19 pandemic, as well as online discussion meetings for the collaborative research projects.
- 4) Organized the Genome Informatics Training Course (GITC) and the BioImage Analysis Training Course (BIATC), both of which were held online.
- 5) 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 2020 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 for prospective students.
- 3) Cooperated the organization of the Freshman Course and the Life Science Retreat as interdepartmental programs in SOKENDAI.
- 4) Gathered and provided useful information for both students and faculty members.

## GENDER EQUALITY PROMOTION GROUP

|                      |                        |
|----------------------|------------------------|
| Professor:           | HIGASHIJIMA, Shin-ichi |
| Associate Professor: | TSUBOUCHI, Tomomi      |

This group manages gender equality promotion activities.

**The group's main activities in 2020 were as follows:**

- 1) *Managed a multi-purpose room for use by researchers with children.*
- 2) *Maintained two group mailing lists for (1) female researchers and (2) researchers with young children in order to share useful information.*

**COLLABORATIVE INNOVATION GROUP**

*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 2020 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

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

Vice-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  
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-Centre for Organismal Studies Heidelberg (COS Heidelberg), Germany

NIBB and COS Heidelberg are conducting collaborative activities based on the academic exchange agreement signed in 2019.

#### *NIBB-COS Heidelberg 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 (p. 74).

#### *The Kick Off (1st) meeting for NIBB–COS Heidelberg International Collaborations*

##### *Lecture series #1 “Plant Root Development”*

The “Kick Off (1st) meeting for NIBB–COS Heidelberg International Collaborations” was held on March 30, 2021. We had planned to hold an NIBB–COS Joint Workshop in Okazaki in 2020, but this was postponed due to the COVID-19 pandemic and was changed to an online exchange program between the two institutes. The program consisted of two events: a lecture series at which researchers from both institutes presented their research projects, and a break-out discussion session using an online communication tool for all members in attendance. The theme of the lecture series was “Plant Root Development”. Dr. Takashi Soyano from the Division of Symbiotic Systems and Dr. Alexis Maizel from COS Heidelberg gave presentations on rhizogenesis and lateral root formation, respectively. 58 members from NIBB and 32 members from COS (both totals include post-docs and students) participated in this event, and lively discussions took place.



At the break-out discussion session, we used the Remo online meeting platform, and the PIs from both sides were

grouped into seven research themes (New Model Organisms, Stem Cells / Regeneration, Development, Cell Biology / Signaling, Neurobiology, Bioimaging, Modeling, and Quantitative Biology) to facilitate discussions. To our delight, this event generated new research ideas and possibilities for collaborative research.

As the overall feedback was very positive, we will continue this format of meetings in 2021, namely the lecture series combined with the break-out discussion session. The theme of the lecture series will change every time to attract a broader range of participants for the lectures as well as for the break-out discussion session.

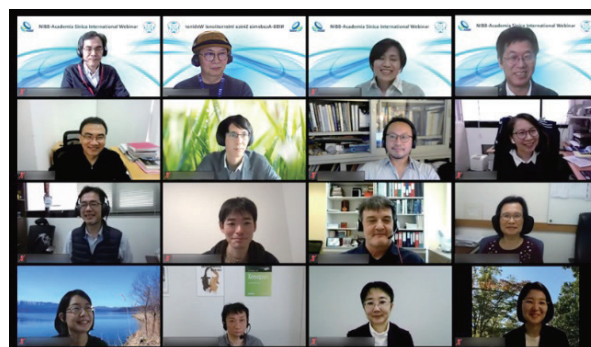
### NIBB-Princeton University, USA

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 AY2020, Dr. Ellen Reed, an IRCC's specially appointed research employee 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. Although it was difficult to travel between NIBB and Princeton University due to the pandemic of COVID-19, collaborative research activities were conducted using online communication tools.

### NIBB–Institute of Cellular and Organismic Biology (ICOB), Academia Sinica, Taiwan

#### *NIBB–ICOB, Academia Sinica, Taiwan, International Webinar of Aquatic Model Organisms for Basic Biology to Human Disease Models*

The “NIBB–Academia Sinica International Webinar of Aquatic Model Organisms for Basic Biology to Human Disease Models” was held online on March 5, 2021. A training course jointly organized by NIBB and Academia Sinica was initially planned in 2020, but was postponed to Autumn 2022 due to the pandemic. In response to this, the course’s organizers held the above-mentioned webinar as an international cooperative activity when international travel was limited. A total of 11 lecturers were invited from China, Singapore, Taiwan, and Japan to attend the online webinar. The lectures covered a wide variety of topics and aquatic



organisms, from corals and sea urchins to amphibians and bony fishes, in the fields of developmental biology, evolutionary biology, genomics, neurophysiology, and immunology.

A total of 100 people from Japan, Taiwan, China, Indonesia, India, Singapore, Germany, Poland, Switzerland, the Netherlands, Ukraine, the United States, Canada, and Argentina registered to this webinar. An advantage of the online format was that the number of registered participants had expanded worldwide. The webinar itself saw roughly 50–60 people consistently staying online to participate in all sessions.

In the post-event questionnaire, 97% of respondents answered that the webinar content was interesting, and many gave high marks to the lecture content and management. During this webinar, we attempted to foster real-time dialogue via Slack in the form of a typed chat format enabling week-long question-and-answer sessions to encourage our participants to discuss the issues. 86% of participants felt that Slack was easy to use, while others preferred direct discussions with all lecturers and participants. In addition, there were useful comments regarding the timing of breaks, which will be helpful in organizing future webinars. We would like to organize scientific events in this format, not only because travel bans remain in effect, but also because this format attracts participants from more diverse countries.

#### Webinar information:

##### Organizers

Kiyoshi Naruse (NIBB / NBRP-medaka / IBBP)  
Ai Shinomiya (ExCELLS / NIBB)  
Yasuhiro Kamei (NIBB)  
Chen-Hui Chen (ICOB, Academia Sinica, Taiwan)  
Sheng-Ping Hwang (ICOB, Academia Sinica, Taiwan)  
Date: March 5, 2021

## Collaborative Activities with Joint Usage/Research Center

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

NIBB and ILTS are conducting collaborative research based on the agreement of cooperation signed in 2019.

#### 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

#### Agreement of Cooperation with IMEG, Kumamoto University

NIBB and IMEG at Kumamoto University have signed an agreement of cooperation, which is deemed effective from May 26, 2020. To prevent the further spread of COVID-19 infection, the signing ceremony was held in a remote capacity via an online connection between Kumamoto and Okazaki.



Dr. Kiyokazu Agata, the Director General of NIBB, and Dr. Hitoshi Niwa, the Director of IMEG, signing the agreement.

This agreement will promote research and education efforts at these two top research institutes, leading the way in advanced research of the highest international standards through mutual collaboration in the fields of developmental medicine and basic biology. The results of the collaborative activities will be used for research on both sides. In addition, the agreement aims to strengthen the support infrastructure for joint usage and joint research nationwide and to revitalize international academic exchange.

Young researchers and graduate students in NIBB and IMEG have organized regular joint mini-meetings focused on developmental biology.



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

### *Agreement of Cooperation with IAMS, Tokushima University*

NIBB and IAMS at Tokushima University have signed an agreement of cooperation, which is deemed effective from November 26, 2020. To prevent the further spread of COVID-19 infection, the signing ceremony was held remotely via an online connection between Tokushima and Okazaki.



Dr. Kiyokazu Agata, the Director General of NIBB, and Dr. Toyomasa Katagiri, the Director of IAMS, signing the agreement.

The aims of this agreement are promotion of mutual collaborative activities in the fields of research related to medical science and basic biology and leading the way in advanced research of the highest international standards. This will promote research and education efforts at these two top research institutes to strengthen the support infrastructure for joint usage and joint research nationwide and to revitalize international academic exchange.

NIBB researchers and IAMS researchers collaborate on several projects using bioimaging and genome editing methods.

## The 14<sup>th</sup> NIBB Bioimaging Forum “A New Aspect of Bioimaging by Non-optical Modality”

Organizing Committee: Shinya Komoto,  
Kagayaki Kato, Joe Sakamoto  
Supervisors: Yasuhiro Kamei, Kiyoshi  
Tatematsu

November 6 (Fri), 2020

The 14<sup>th</sup> NIBB Bioimaging Forum supported by Advanced Bioimaging Support (ABiS) and entitled “A New Aspect of Bioimaging by Non-optical Modality” was held on November 6, 2020.

NIBB Bioimaging Forums have previously focused on optical observation techniques such as super-resolution microscopy using adaptive optics, and the imaging of biophysical properties. Therefore, during the 14<sup>th</sup> version of this event, we focused on Computed Tomography (CT) especially through the utilization of X-ray, synchrotron radiation and magnetic resonance, as a non-optical observation method instead. CT is a powerful tool for the visualization of the three-dimensional structure of samples without destroying them. Nevertheless, it is difficult for newcomers to engage in research due to the embryonic nature of the existing CT user community, and the difficulty of accessing devices. Therefore, we held this forum to provide opportunities for communication with both existing and potential CT users, as well as to share information.

When people hear the term CT, many imagine machines in hospitals that medically diagnose humans. However, CT is not used only in the medical field, but also in various non-medical fields, such as industry, biology and so on. Therefore, we invited lecturers from both academia and company. Lecture topics were diverse and included areas such as sample preparation, observation methods, recent trends in CT device, and applications to academic research.

Although the forum was held on-site in 2019, it was postponed because COVID-19 outbreak. The organizing committee subsequently decided to merge the 2019 and 2020 versions of the event and hold the forum online using the Zoom online meeting platform. To organize and manage the form both quickly and effectively, the three organizers gathered at NIBB to live stream the forum. Communication difficulties were experienced by some participants, among several other things that needed to be improved. In contrast, lecturers who could not attend on the day of the forum took advantage of the format and gave their presentation by pre-recording it and streaming it on the day of the event.

In closing, I would like to express my appreciate to all participants and organizers, and hope that the forum was helpful for the former’s research,

(Joe Sakamoto)

## 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 2020, 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, we initially held a practical course on RNA-seq analysis, that had been postponed due to the COVID-19 pandemic, only. However, we subsequently held a full version of the training course. All courses were held online to prevent the spread of COVID-19 infection.

### 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)

June 4 (Thu)–5 (Fri), 2020

(Practical Course) RNA-seq Analysis Pipeline

- 33 participants (including 1 from NIBB)
- Program:
  1. Introduction to RNA-seq
  2. NGS Basic Data Format and Basic Tools
  3. Visualization of NGS Data
  4. 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

November 26 (Thu)–27 (Fri), 2020

- 32 participants and 9 auditors (including 3 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

### Introduction to RNA-seq: RNA-seq Analysis Pipeline

March 10 (Wed)–11 (Thu), 2021

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

- 31 participants and 10 auditors (including 2 from NIBB)

## The Bio-imaging Data Analysis Training Course 2020

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

December 9 (Wed)–11 (Fri), 2020

The 8th 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. 114 people applied for the course, which had an announced capacity of 16 participants. Given the high demand for courses on these subjects, we accepted 18 participants.

This course's lectures were conducted with the aim of guiding participants towards 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 microscopy 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, and Assoc. Prof. Shoji MANO). General support provided includes budget planning and management of ABiS activities. In particular, we promote ABiS activities via its associated website and other media, so that KAKENHI researchers make full use of the ABiS platform to accelerate their research projects. In addition, we organize technology training sessions, workshops, and symposia to disseminate advanced imaging technologies and share information about them.



We also coordinate with the other three platforms (Platform of Supporting Cohort Study and Biospecimen Analysis, Platform of Advanced Animal Model Support, and Platform for Advanced Genome Science) to provide both multidisciplinary and international support.

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

GBI also aims to establish an image data sharing infrastructure system, promote academic/industrial cooperation and formulate a global strategy for bioimaging research. The GBI event “The Fifth Exchange of Experience (EoE V)” to be hosted by ABiS, was initially planned to be held in Okazaki during autumn of 2020, but was held online instead to prevent the spread of COVID-19.

## ABiS Symposium “The Present and Future of Advanced Bioimaging -Research Strategy in Japan-”

February 24 (Wed), 2021

The ABiS symposium “The Present and Future of Advanced Bioimaging -Research Strategy in Japan-” was held as an online event, and saw four researchers, who have received support from ABiS in the past, introduce their research and achievements which themselves were aided by the aforementioned organization. Another four researchers, who currently study cutting-edge life science via the application of new imaging technologies, also gave additional lectures. On top of this, a panel discussion entitled “Promotion of Bioimaging Research and Support” was carried out to discuss the future of bioimaging in Japan (Figure 1).



Figure 1. Lectures and chairpersons of ABiS Symposium “The Present and Future of Advanced Bioimaging -Research Strategy in Japan-”.

## 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 FY2020, due to the global spread of COVID-19 and subsequent attempts at preventing further infection, this program mainly accepted international students attending universities in Japan. Consequently, two international students, one from the Philippines and the other from the United Kingdom, were selected. Because of the situation in Japan, one of them came to NIBB in March 2021, and the other will come during FY 2021. Furthermore, we received applications from two other students who belonged to overseas universities and were staying in Japan due to circumstances unrelated to the Internship Program; one of whom was subsequently selected and stayed at NIBB for two months.

### Report from a participant Sea Hee Yook Imperial College London

I immersed myself in the NIBB internship program as an undergraduate student who had just completed her first year of a Medical Biosciences degree at Imperial College London. Although I had limited experience in conducting experiments in a professional laboratory, I was motivated to intern at Professor Aoki's laboratory specializing in quantitative biology upon reading one of his publications. Among the other host labs, Professor Aoki lab's unique approach in presenting scientific results through microscopy visualization and precisely quantified data appealed to me as an opportunity to obtain skills in both practical research and scientific communication. Throughout the course of the next month and a half, I was able to get a deep insight into using advanced techniques and equipment to visualize and model various intracellular mechanisms.

My research primarily focused on examining the proteins involved in controlling the cell cycle of *Schizosaccharmyces pombe*, otherwise known as fission yeast. The progression of biochemical events from G1 to M phase in its cell cycle is known to be regulated through a single CDK-cyclin complex composed of Cdc2 and Cdc13, alongside the activation of additional protein substrates at distinct timepoints. To

examine such properties, I conducted confocal fluorescence imaging and quantitative analysis of the images.

Whilst being engaged in using various imaging tools, I was also introduced to basic laboratory techniques such as western blotting, transforming plasmids into cell lines, designing PCR primers, and conducting swift but precise DNA work. Additionally, I learned about the importance of performing detailed experimental planning, careful observation, interactive discussions with other researchers, reliable data analysis, and effective troubleshooting to develop as a well-established researcher capable of presenting influential scientific discoveries.

Undoubtedly, the internship program provided me with an opportunity to further explore Japanese culture by encountering people with different values, interests, and lifestyles. I want to thank the NIBB for providing me with such an invaluable opportunity, and the people at Professor Aoki's laboratory for their unconditional support throughout the internship. I am confidently returning home with newly acquired skills and attributes that have equipped me to continue pursuing my passion for research.



## 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 Junior High Schools

NIBB, in collaboration with the Okazaki City Board of Education, visited junior high schools during 2020 to give special classes.

**November 2 (Mon), 2020**

*Special class at Ryukai Junior High School*

■ Director General Kiyokazu Agata



**November 5 (Thu), 2020**

*Special class at Tokai Junior High School*

■ Prof. Teruyuki Niimi



**November 11 (Wed), 2020**

*Special class at Ryunan Junior High School*

■ Prof. Teruyuki Niimi



**November 12 (Thu), 2020**

*Special class at Kawai Junior High School*

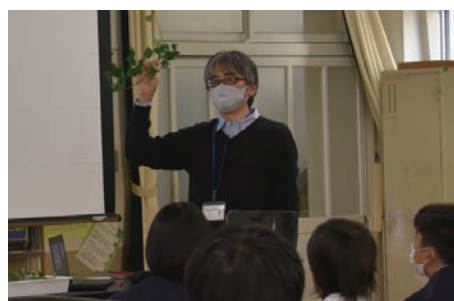
■ S. A. Prof. Kiyoshi Naruse



**November 17 (Tue), 2020**

*Special class at Shinkayama Junior High School*

■ S. A. Assoc. Prof. Kenji Takizawa



**December 1 (Tue), 2020**

*Special class at Aoi Junior High School*

■ Prof. Teruyuki Niimi



**December 4 (Fri), 2020**

*Special class at Mutsumi Junior High School*

■ Assist. Prof. Kazuo Tsugane





## Visit to High Schools

February 5 (Fri), 2021

*Special class at Okazaki High School*

■ Prof. Takashi Ueda



August 4 (Tue), 2020

*Special class at Okazaki Kita High School*

■ Assist. Prof. Hiroki Takahashi



December 7 (Mon), 2020

*Special class at Okazaki Kita High School*

■ Prof. Jun-ichi Nakayama



March 16 (Tue), 2021

*Special class at Okazaki Kita High School*

■ Dr. Gergo Palfalvi



## Seminar for school teachers

February 9 (Tue), 2021

■ Prof. Shinji Takada



## Feedback session regarding research presentations by high school students

August 28 (Fri), December 25 (Fri), 2020

■ S. A. Assist. Prof. Kiyoshi Tatematsu



## Programs broadcast live online

June 13 (Sat)-20 (Sat), 2020

*“Watching Both the Laying of Medaka Eggs and Their Hatching”*

This was a 200 hour live broadcast displaying the process entailed in the birth of medaka ranging from egg laying to hatching. Commentaries were provided by researchers studying medaka via the live internet broadcast platform, NicoNico

■ S. A. Prof. Kiyoshi Naruse

■ Dr. Tadashi Sato

■ S. A. Assist. Prof. Tomoko Kurata



August 9 (Sun)–16 (Sun), 21 (Fri), 2020

*"Watching the Planarian Regeneration Process over the Course of 200 Hours"*

This was a 200 hour live broadcast displaying the process of planarian regeneration. Commentaries were provided by researchers studying planarians via the live internet broadcast platform, NicoNico

- Director General Kiyokazu Agata
- S. A. Assist. Prof. Tomoko Kurata



Lectures for the Public

February 23 (Tue), 2021

Online lecture at Natural History Museum/Sea of Life-

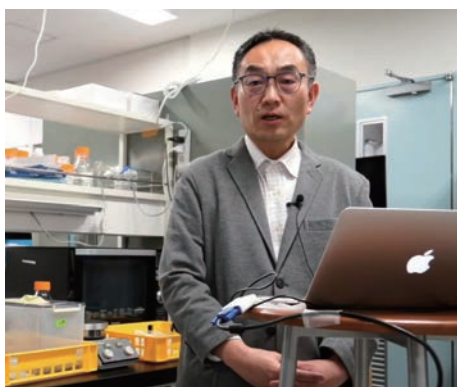
- Dr. Tatsuhiro Konagaya
- Mr. Yasuhiko Chikami



February 28 (Sun), 2021

Science Day in Toki (Online Lecture)

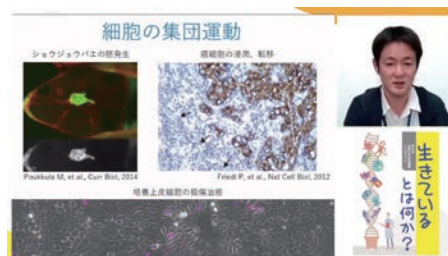
- Prof. Teruyuki Niimi



March 13 (Sat), 2021

*The 31st National Institutes of Natural Sciences Symposium*

- Prof. Kazuhiro Aoki

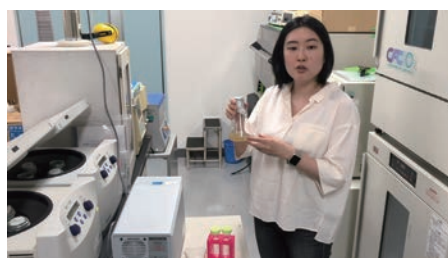


June 14 (Sun), 2020

National Institute of Natural Sciences

*The 9th Young Researcher Award Commemorative Lecture*

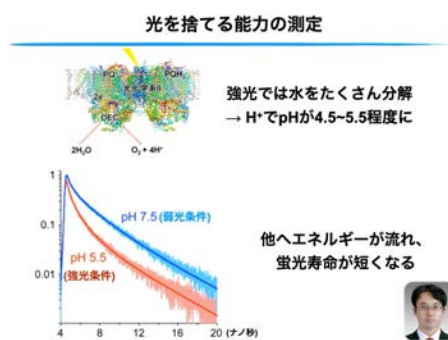
- S. A. Assist. Prof. Yoko Otsubo



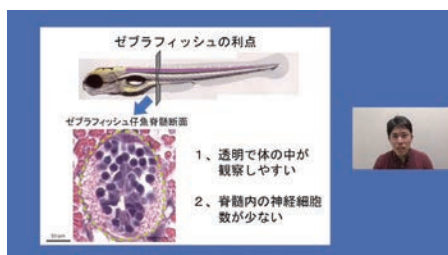
February 24 (Wed), 2021

Presentation by OKASHIN (Okazaki Credit Union) scholarship students

- Mr. Akimasa Watanabe



- Mr. Kohei Kawano







## Access



### From Tokyo

Take the Tokaido Shinkansen and get off at Toyohashi, change to the Meitetsu Line and get off at Higashi Okazaki Station (approximately 20 minutes from Toyohashi).

### From Osaka

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

### From Central Japan International Airport

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

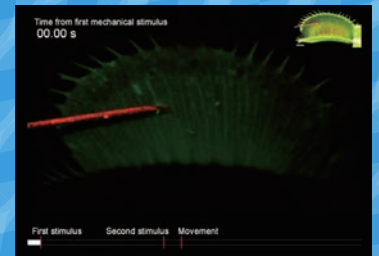


### From Higashi Okazaki Station to Each Area

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

### By Car

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



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

tel: +81 564-55-7000 fax: +81 564-53-7400

<https://www.nibb.ac.jp/en/>

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