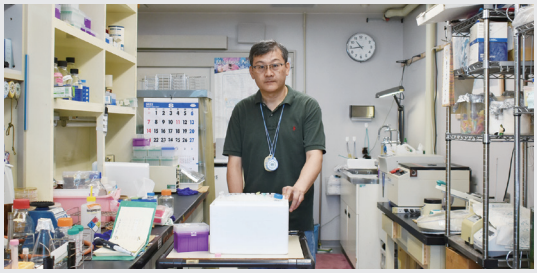
LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA, Yoshiaki*



Tor (target of rapamycin) is a Ser/Thr protein kinase which is well conserved in organisms spanning from yeasts to mammals. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. On one hand, TORC1 is involved in amino acid sensing, regulation of protein synthesis (especially the translation step), the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. Up until now, it's been deemed unlikely that TORC2 can recognize nutrient signals.

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

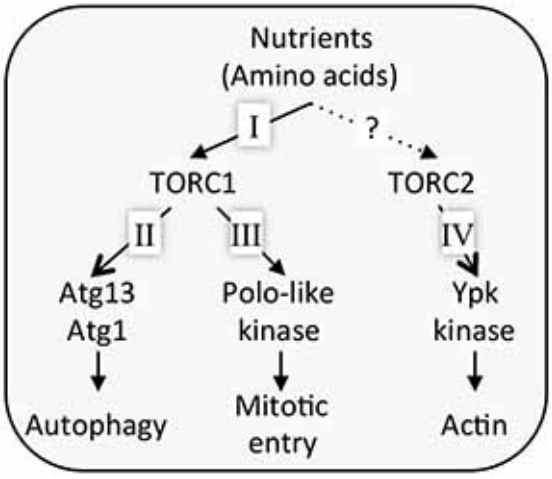


Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

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

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

Based on these results, a TORC1 regulatory model was proposed which contends that free tRNA released from protein synthesis under amino acid starvation inhibits TORC1 activity. Therefore, TORC1 employs a tRNA-mediated mechanism to monitor intracellular amino acids (Figure 2).

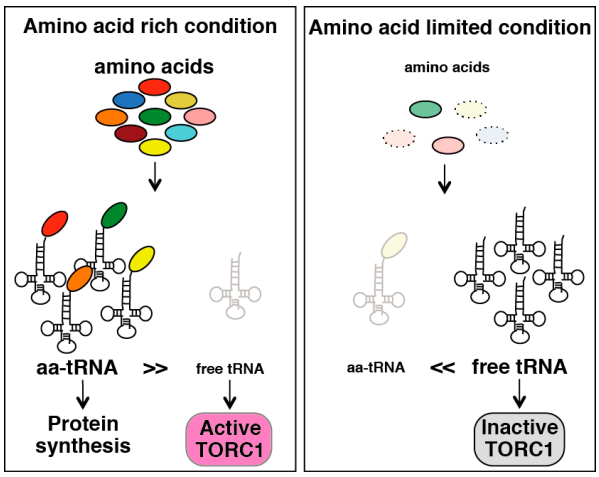


Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

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

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

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

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

III. Relationships between TORC1 and eIF4E-binding proteins

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

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

Publication List:

〔**Original paper**〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

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

Genomic structures and their genetic information are stably transmitted into daughter cells and future generations during cell division; however, they can vary genetically and/or epigenetically. Such variability impacts gene expression and evolution. To understand these genomic dynamics in eukaryotes, particularly in plants, we are analyzing the flower pigmentation of morning glories, including *Ipomoea nil* (Japanese morning glory), *I. purpurea* (the common morning glory) and *I. tricolor*.

I. Flower pigmentation patterns

The wild type morning glories produce flowers with a uniformly pigmented corolla. For our study, we collected mutants displaying pigmentation patterns. Flower pigmentation patterns are easily observable and the molecular mechanisms underlying these phenomena provide useful model systems for investigating genomic variability.

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

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

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

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

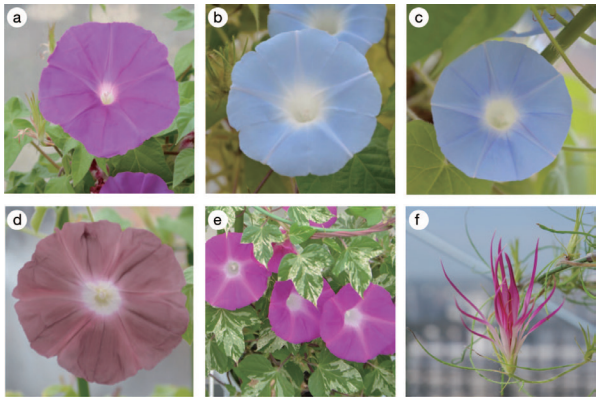


Figure 2. The *I. nil* lines used for the whole-genome analysis. (a) Violet: The most widely used standard line. (b) Africa: The wildtype line isolated in Guinea in 1956. (c) Nepal: The wildtype line isolated by Dr. Sasuke Nakao in Nepal in 1952. (d) Danjuro, one of the most popular cultivars, named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

III. Flower opening and time control

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

IV. Morning glory bio-resources

NIBB is the subcenter for the NBRP dedicated to morning glories. In this project, we collect, maintain, and distribute standard and mutant lines for flower pigmentation and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* is one of the most popular floricultural plants in Japan and has a 100-year history of extensive related genetic studies. Our collection includes 241 lines and 177,000 DNA clones. The whole-genome sequence, the transcriptome

sequences, and the end sequences of the DNA clones can be viewed via the *I. nil* genome database (http://viewer.shigen.info/asagao/index.php).

Publication List:

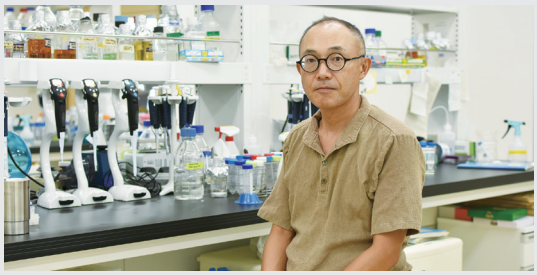
〔Original paper〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*



Although transposons occupying a large portion of the genome in various plants were once thought to be junk DNA, they play an important role in genome reorganization and evolution. Active DNA transposons are important tools for gene functional analysis. The endogenous non-autonomous transposon, nDart1, in rice is said to generate various transposon-insertion mutants because nDart1 elements tend to insert into genic regions under natural growth conditions. The transpositions of nDart1 were promoted by an active autonomous element, aDart1-27, on chromosome 6. By using the endogenous nDart1/aDart1-27 system in rice, a large-scale nDart-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation. The nDart1/aDart1-27 system was introduced into a rice variety, Koshihikari (*Oryza sativa* subsp. *japonica*), and Basmati (*Oryza sativa* subsp. *indica*). Various mutations caused by the insertion of nDart have been screened for characteristic phenotypes.

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

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

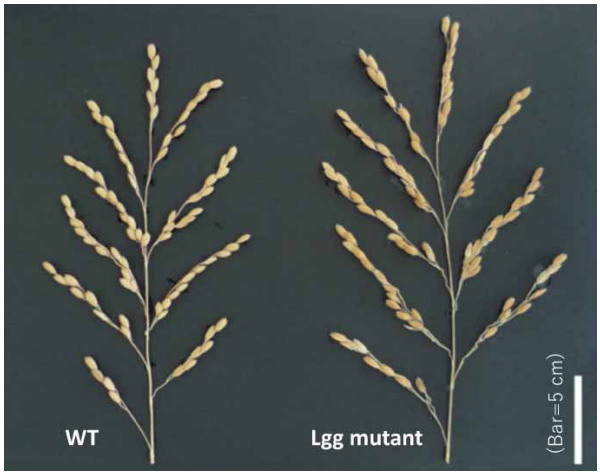


Figure 1. Phenotype of Large gain (*Lgg*). Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

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

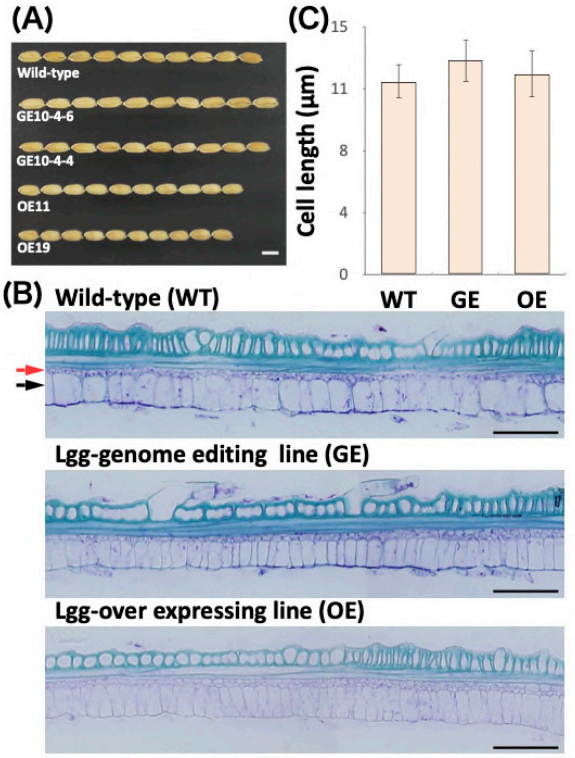


Figure 2. The spikelets morphology and longitudinal sections and cell length of the lemma of NP, GE, and OE, respectively. (A) The spikelets. Bar = 5 mm. (B) The Section of lemma in transgenic plants. The red and black arrows indicate adaxial epidermis and chlorophyll-contained parenchyma cell. (C) Cell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

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

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

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

I. Cell segmentation/tracking system

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

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

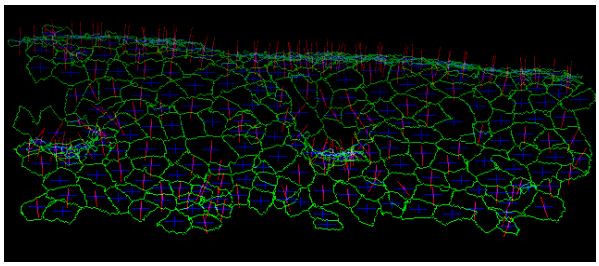


Figure 1. Visualized apical cell surface of Drosophila embryonic epidermal cells. A time-lapse confocal microscopic data set expressing E-cadherin-GFP was processed. Cell boundaries (green), the center of gravity (blue), and normal vector (magenta) are indicated for each cell.

II. Image processing pipeline for morphogenesis

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

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

III. Quantification of fish body shape

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

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

Publication List:

〔Original paper〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

OHTA Group

Specially Appointed Assistant Professor:
OHTA, Yusaku

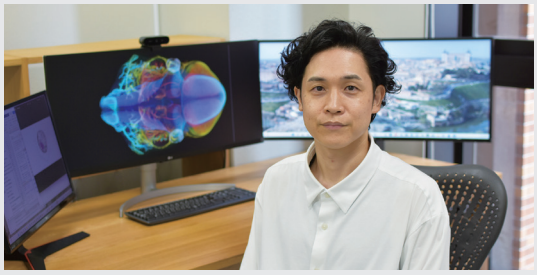


Image analysis is an important component of understanding life sciences. It enables us to quantify phenomena by extracting meaningful information from a large number of images and to properly represent that information. I am developing image analysis techniques aimed at analyzing the developmental process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

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

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

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

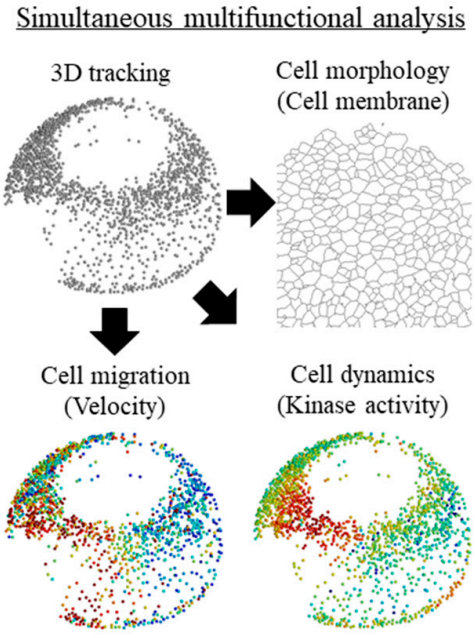


Figure 1. Simultaneous multifunctional analysis of early embryonic development

II. Research support by image analysis

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

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

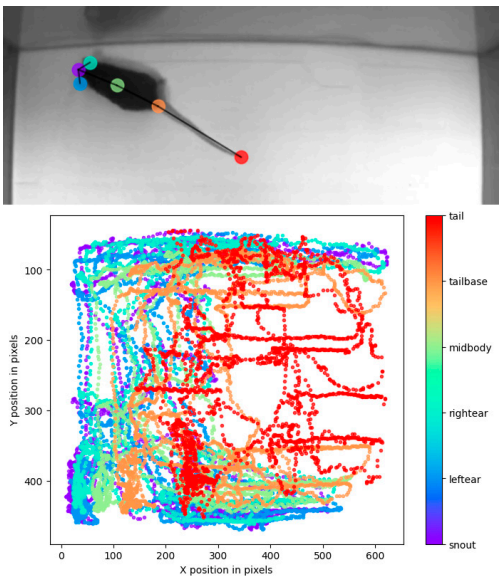
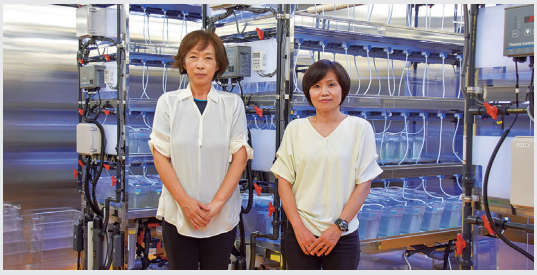


Figure 2. A website that explains how to use image analysis software

LABORATORY OF BIOLOGICAL DIVERSITY

SHINOMIYA Group

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



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

Medaka (*Oryzias latipes*) provide an excellent model to study these mechanisms because of their rapid and obvious seasonal responses. In addition, it is also possible to apply transgenic and genome-editing approaches when researching them, as well as reference genome sequences.

In keeping with this, we are currently using Medaka to study the molecular mechanisms of seasonal adaptation, as well as the relationship between seasonal information from the environment and the organism's response.

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

I. Underlying mechanisms that define critical day length and temperature

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

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

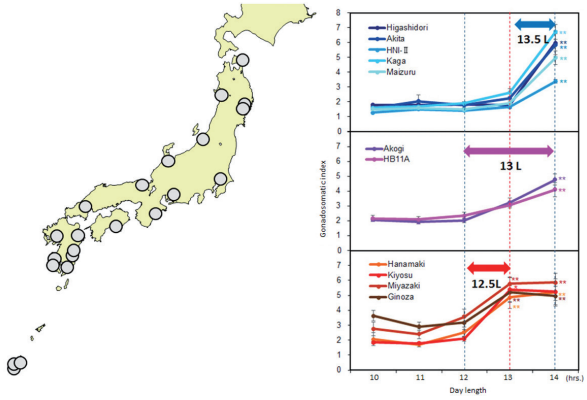


Figure 1. Differences in critical day length (right) were detected among medaka populations that had originated in various latitudes (left) .

Geographical variation critical day length was detected, and populations from lower latitudes indicated a shorter critical day length (Figure 1). To identify the genes governing critical day length, quantitative trait loci (QTL) analysis was conducted using F_2 offspring derived from crosses between populations experiencing different critical day length. We thus identified significant QTLs using Restriction-site Associated DNA (RAD) markers (Figure 2).

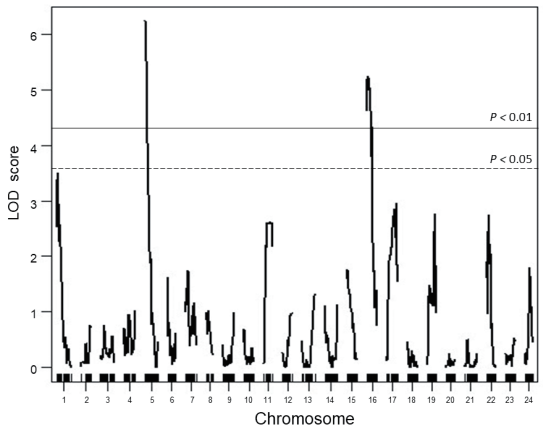


Figure 2. Result of QTL analysis for critical day length.

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

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

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

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

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

Publication List:

〔**Original papers**〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

SAITO Group

Specially Appointed Associate Professor
SAITO, Nen

Postdoctoral Fellow: *FUKUYAMA, Tatsuya*

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

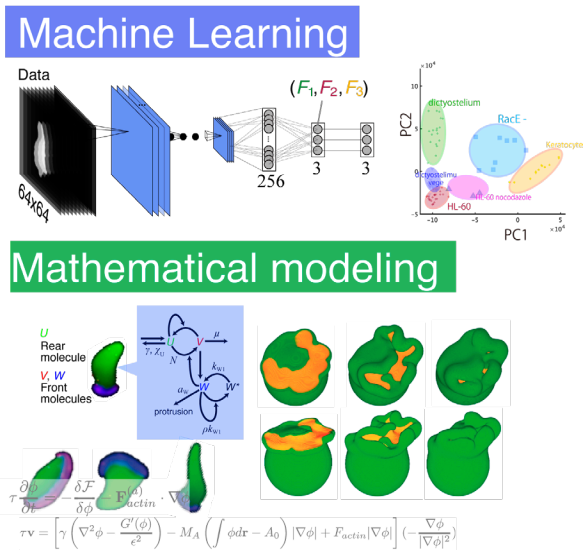


Figure 1. Researches combining machine learning and mathematical modeling.

I. Simulations of amoeboid cells

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

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

II. Mathematical Model of Microbial Symbiosis via Metabolite Leakage

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

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

III. Mathematical Model of collective cell migration

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

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

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

Publication List:

〔**Original papers**〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

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

I. Gonadotropins in the starfish, *Patiria pectinifera*

Gonadotropins play important regulatory roles in reproduction in both vertebrates and invertebrates. The vertebrate gonadotropins, LH and FSH, are structurally and functionally conserved across various species, whereas no such molecule has been identified in invertebrates. Some gonadotropic hormones have been found in invertebrate species. An insulin-like peptide was reported to be responsible for the regulation of egg maturation in the mosquito, *Aedes aegypti*, thus demonstrating the involvement of insulin signaling in egg maturation among invertebrates.

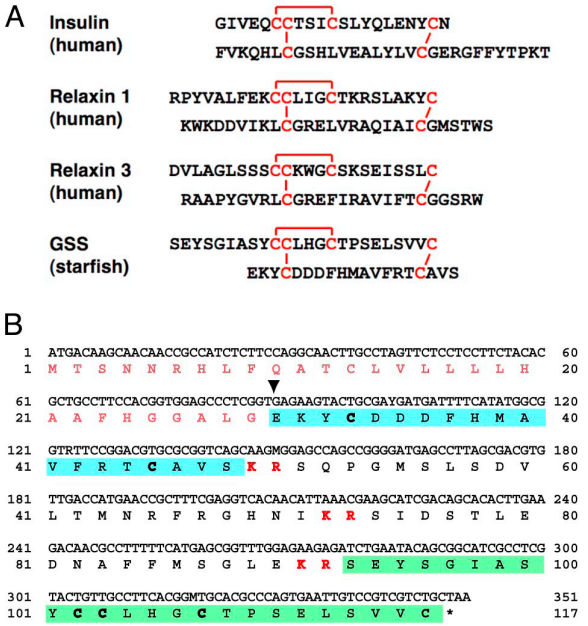


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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

II. Search for reproductive hormones in echinoderms

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

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. In particular, relaxin like peptide precursor genes and insulin/IGF like peptide precursor genes were identified from many species. We are producing these neuropeptides in a chemical synthetic manner to provide to collaborating researchers for biological assays.

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

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

Publication List:

〔Original papers〕

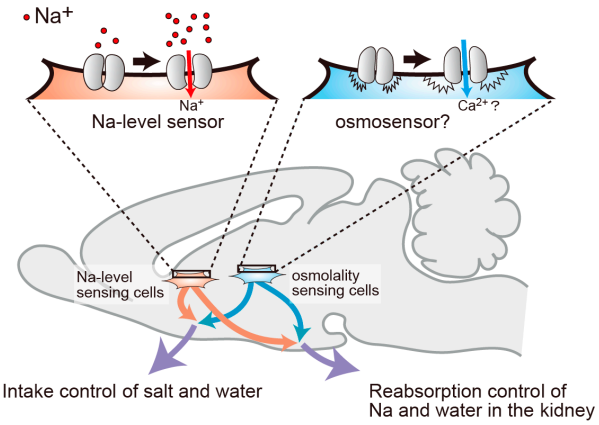
- Nishiike, Y., Miyazoe, D., Togawa, R., Yokoyama Keiko and Nakasone, K., Miyata, M., Kikuchi, Y., Kamei, Y., Todo, T., Ishikawa-Fujiwara, T., Ohno, K., Usami, T., Nagahama, Y., and Okubo, K. (2021). Estrogen receptor 2b is the major determinant of sex-typical mating behavior and sexual preference in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

LABORATORY OF BIOLOGICAL DIVERSITY

SAKUTA Group

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

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



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

I. Thirst control by Na_x and TRPV4

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

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

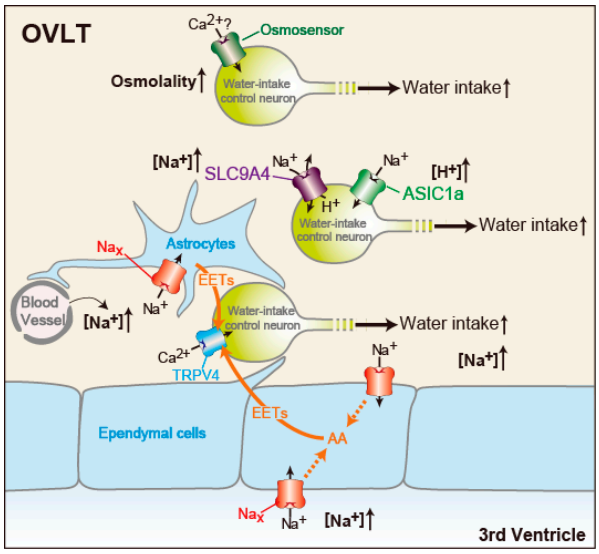


Figure 1. Schematic drawings of sensing mechanisms of body-fluid conditions in the OVLT responsible for the induction of water intake. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

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

LABORATORY OF BIOLOGICAL DIVERSITY

WUDARSKI Group

Specially Appointed Assistant Professor:
WUDARSKI, Jakub

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

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

Positional control of regeneration in flatworms

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

The research is financed by the Mistubishi Foundation grant.

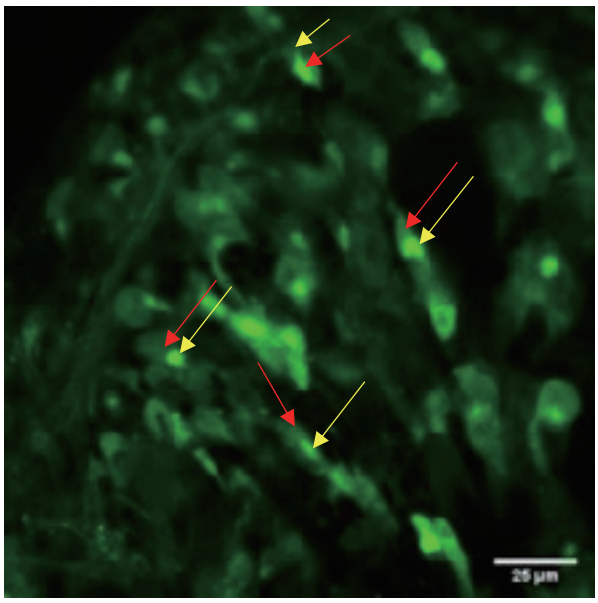


Figure 1. ERK-KTR biosensor in *Macrostomum lignano* red arrow points at the cytoplasm and yellow at the nucleus.

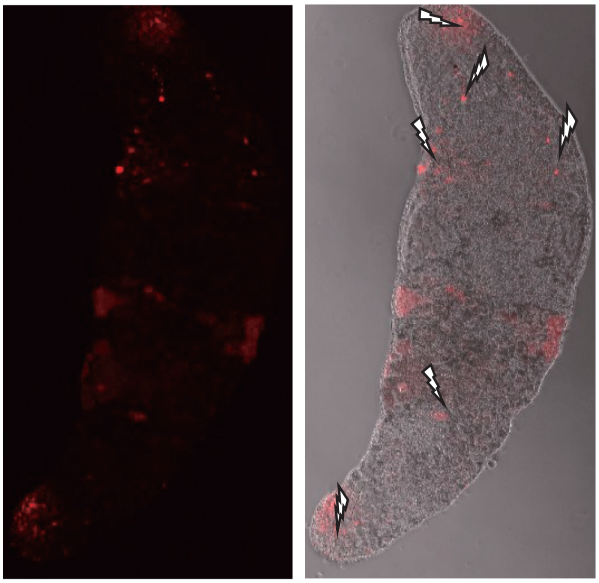


Figure 2. Expression of mScarlet under the HSP20 promoter 24 hours after induction using IR-LEGO. The lightning bolts point at the targeted sites.

Publication List:

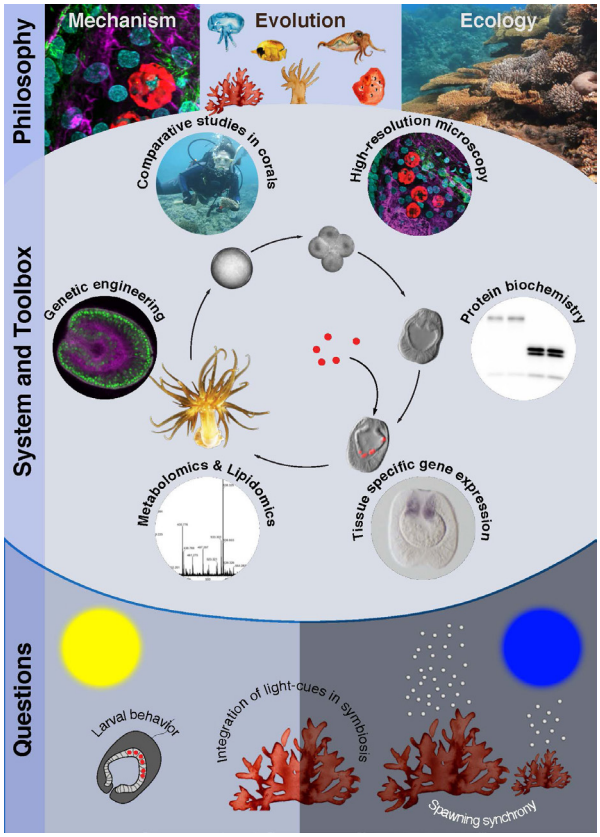
〔Original paper〕

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

NIBB-COS International Collaboration Research Project

GUSE Group

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



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

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

I. Light sensing changes by symbiotic status

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

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

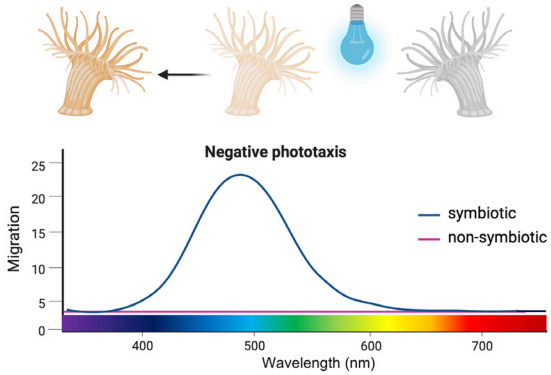


Figure 1. Wavelength specificity of negative phototaxis in symbiotic Aiptasia.

II. Functional analysis of Aiptasia opsins

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

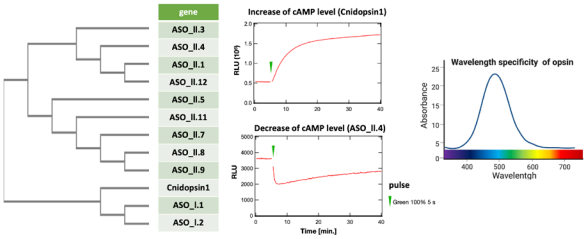


Figure 2. Functional analysis of Aiptasia opsins cloned in this study.

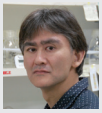
Publication List:

〔Original paper〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

YAMASHITA AND OTSUBO Group



Specially Appointed Associate Professor
YAMASHITA, Akira

Specially Appointed Assistant Professor: *OTSUBO, Yoko*
Technical Assistant: *NAKADE, Atsuko*
Admin Support Staff: *SAKAGAMI, Mari*

Cells sense the environment around them (*e.g.* the amount of nutrients and hormones present, as well as the temperature and pressure), and decide what kind of activities to undertake based on this information. In response to ambient conditions, germ cells producing sperm and eggs begin halving their number of chromosomes during a special kind of cell division called meiosis; a process for bringing forth genetically diverse progeny. In our group, we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis (Figure 1), to research the mechanisms by which cells switch from mitosis (a kind of cell division that divides cells equally to create two identical cells) to meiosis. In addition, we have started to study cellular responses to a novel stress: atmospheric pressure plasma.

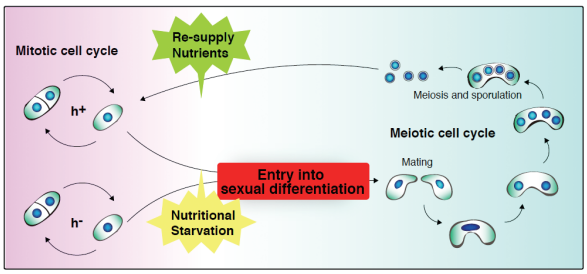


Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting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

I. Signaling pathways that regulate the onset of sexual differentiation

We have been trying to elucidate how *S. pombe* cells switch their cell cycle mode from mitotic to meiotic. To achieve this, we have focused on a highly conserved kinase, namely Target of Rapamycin (TOR) kinase, which plays a key role in the recognition of nutrition and the onset of sexual differentiation in *S. pombe*. TOR kinase forms two types of complexes, TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit, and is essential in suppressing sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for the onset of sexual differentiation under nitrogen starved conditions.

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich mediums under restrictive temperature conditions. To gain an insight into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions, as *tor2* mutants do. We designated these mutants as *hmt*, which stands for hypermating and temperature-sensitive growth. We cloned the responsible genes and found that several responsible genes encoded tRNA-related factors. We also found that the expression of tRNA precursors decreases upon nitrogen starvation occurring. Furthermore, overexpression of tRNA precursors prevents TORC1 downregulation in response to nitrogen starvation and represses the initiation of sexual differentiation. Based on these observations, we have proposed that tRNA precursors act as key signaling molecules in the TORC1 pathway in response to nitrogen availability (Figure 2). We are also studying how *S. pombe* cells respond to other types of starvation other than that of nitrogen.

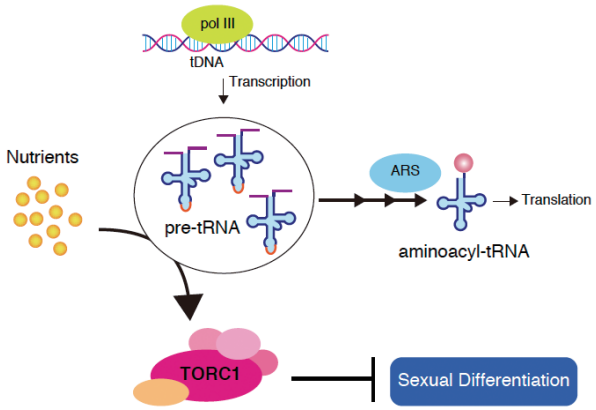


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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

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

A YTH-family RNA-binding protein Mmi1 plays a crucial role in the selective elimination system of meiosis-specific transcripts during the mitotic cell cycle. Mmi1 recognizes a region termed DSR (Determinant of Selective Removal) in meiotic transcripts, which is enriched by repeated hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machinery. Mmi1 also induces the formation of facultative heterochromatin at a subset of its target genes. Furthermore, Mmi1 regulates the termination of transcription of its target genes. Mmi1-mediated termination of an upstream non-coding RNA ensures the expression of downstream genes, one of which encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) essential for the initiation of sexual differentiation.

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

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

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

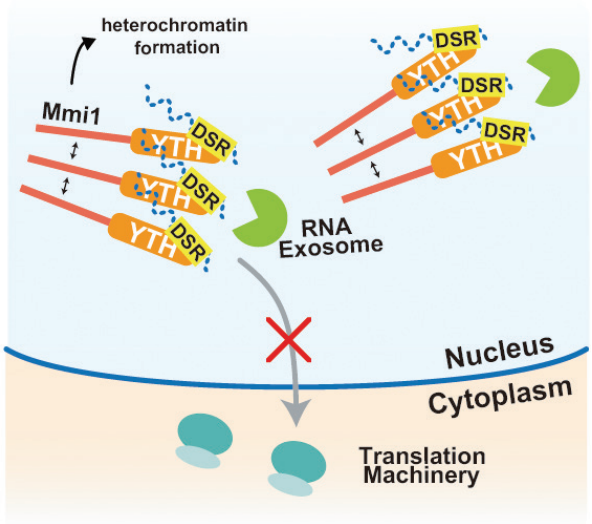


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

III. Cellular responses to atmospheric pressure plasma

Plasma, which is the fourth state of matter after solid, liquid and gas states, is a form of ionized or electrically charged gas. It has been known that plasma irradiation induces a wide variety of effects on living organisms. Recently, atmospheric pressure plasma has been used in various fields including medicine and agriculture. However, detailed mechanisms underlying responses to plasma irradiation remains unknown. Accordingly, we are addressing molecular mechanisms of cellular responses to plasma by using the fission yeast *S. pombe*. To this end, we have developed plasma irradiation devices that enable control of gas temperature in collaboration with the National Institute for Fusion Science (Figure 4).

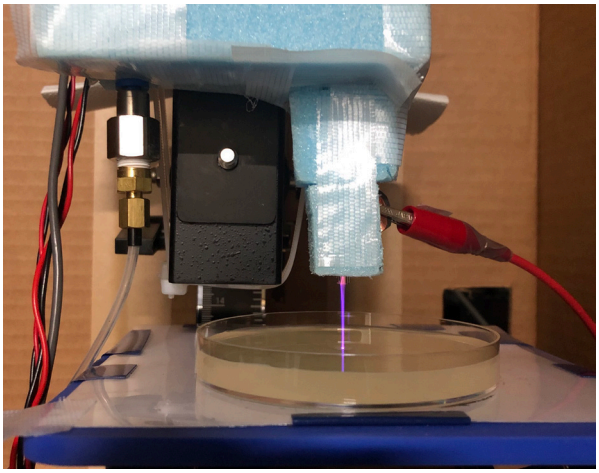


Figure 4. Direct plasma irradiation to the fission yeast *S. pombe* on an agar medium.

Publication List:

〔Original paper〕

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

NINS ASTROBIOLOGY CENTER

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

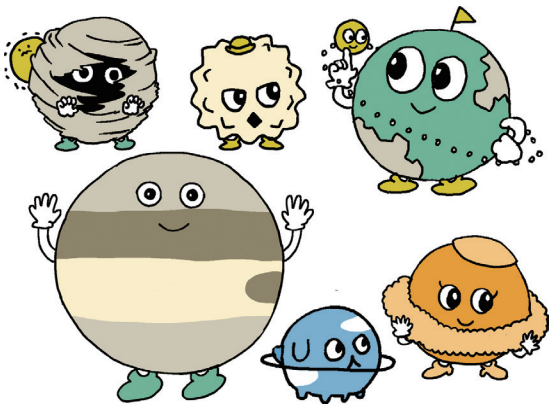


Figure 1. ABC mascot characters: "Exoplanet Friends" (Credit: Hayanon Science Manga Studio)

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

LABORATORY OF BIOLOGICAL DIVERSITY

TAKIZAWA Group

Specially Appointed Associate Professor
TAKIZAWA, Kenji

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

Light adaptation mechanisms of photosynthesis

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

Characterize hypothetical plants in exo-planets

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

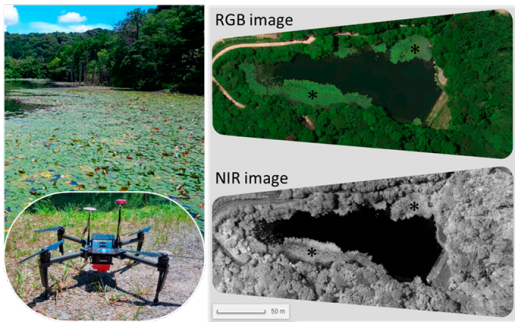


Figure 2. Remote sensing of light reflectance of floating leaf of waterlily. Light reflectance in visible light (RGB) and NIR were obtained by the drone-based multiband sensor.

Publication List:

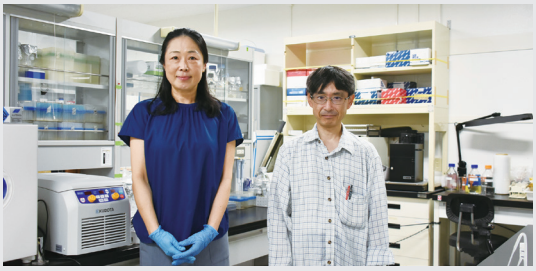
〔Original papers〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

FUJITA Group

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



In nature, a variety of self-organized patterns, such as the galaxy and the snowflake, are found on a wide range of spatiotemporal scales. Particularly in living organisms, such self-organization of spatiotemporal patterns is both remarkable and essential. Therefore, we aim to elucidate the mechanism of generation and control of self-organized patterns in living systems with a particular focus on plants using both theoretical and computational approaches (Figure 1).

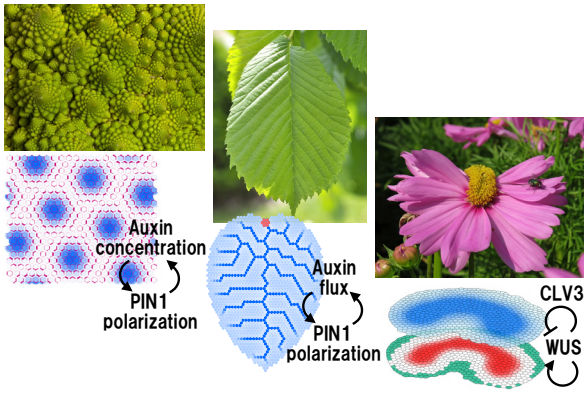


Figure 1. (Left) Phyllotaxis pattern is self-organized by the feedback regulation between auxin concentration and PIN1 polarization. (Middle) Leaf venation pattern is self-organized by the feedback regulation between auxin flux and PIN1 polarization. (Right) Shoot apical meristem (SAM) is self-organized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

I. Spatiotemporal self-organization of cell population

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

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

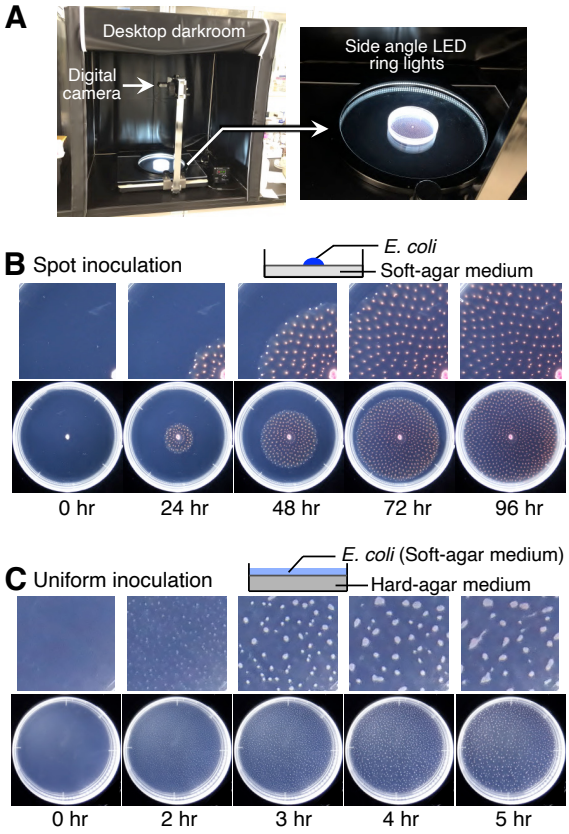


Figure 2. (A) A device system for photographs and time-lapse videos of spatiotemporal patterns of cell populations. (B) Spot inoculation; *E. coli* cells are inoculated in the center of soft-agar plates. The cell population spreads outward while forming a stable spot pattern. (C) Uniform inoculation;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

Publication List:

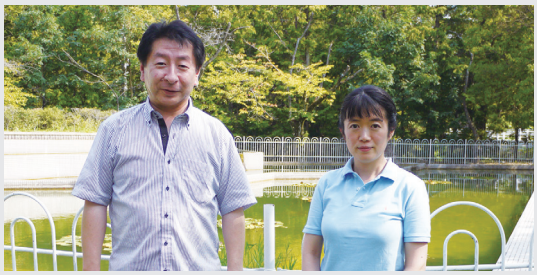
〔Original paper〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

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



I. Condensin-dependent chromatin folding

Chromosome condensation is a basic cellular process that ensures the faithful segregation of chromosomes in both mitosis and meiosis. Any abnormality in this process leads to segregation errors or aneuploidy, which results in cell death. Chromosome condensation is mainly achieved by condensin, a hetero-pentameric protein complex, widely conserved across a variety of organisms ranging from yeast to humans. Despite its conservation and importance in chromosome dynamics, it is not fully understood how condensin works.

Our aim is to understand the mechanism and regulation of chromosome condensation. To this end, we have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. To date, the best characterized condensin binding region is the rDNA repeat on the right arm of chromosome XII in budding yeast. We further discovered the multiple protein network required to recruit condensin to the RFB site.

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

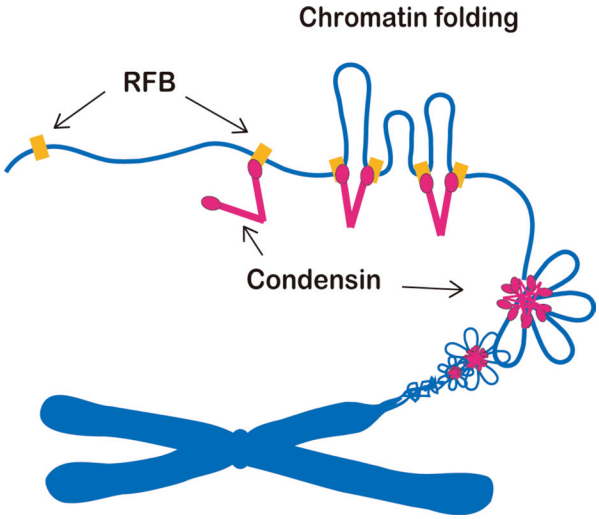


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

II. Plasma-induced cellular responses

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

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

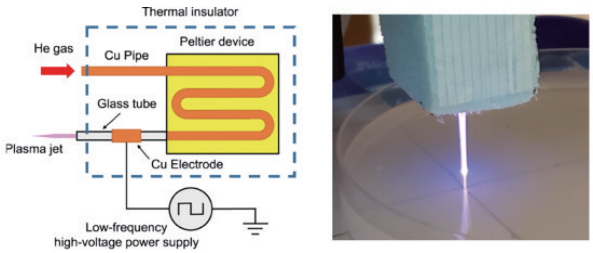


Figure 2. Schematic diagram of atomosperic pressure plasma irradiation device (left). Direct plasma irradiation experiment (right).

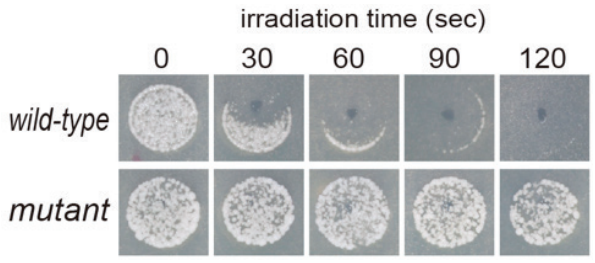


Figure 3. wild-type (upper panels) and plasma-resistant mutant (lower panels)