

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).

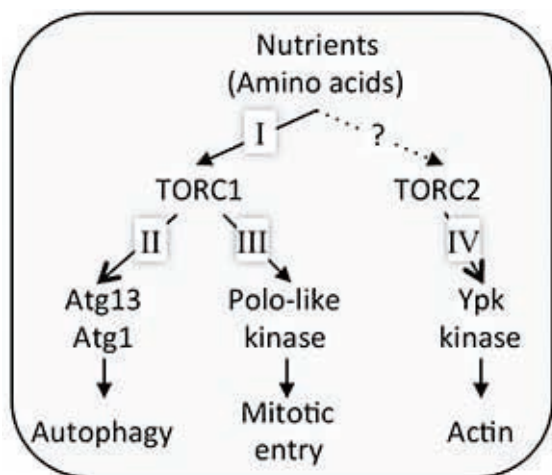


Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

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).

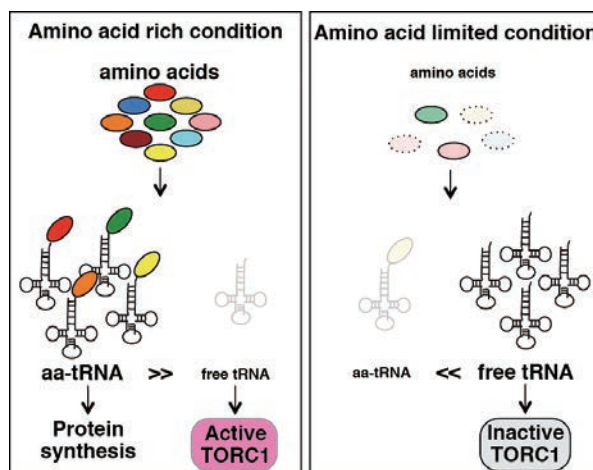


Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

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:

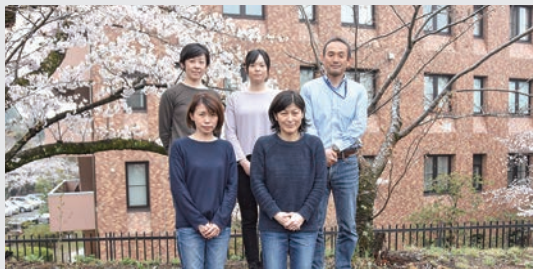
[Original paper]

- 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.

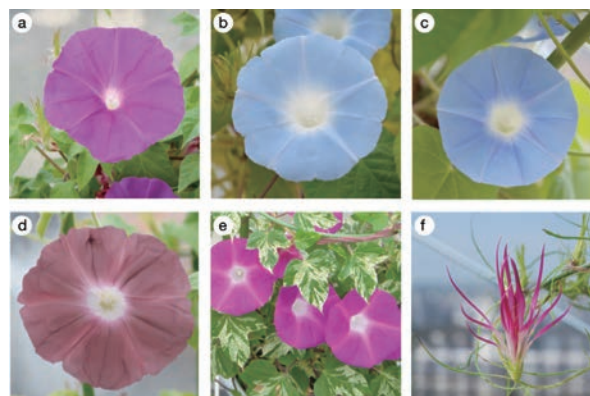


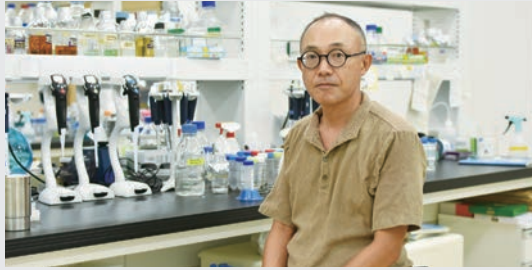
Figure 2. The *I. nil* lines used for the whole-genome analysis. (a) Violet: The most widely used standard line. (b) Africa: The wild-type line isolated in Guinea in 1956. (c) Nepal: The wild-type line isolated by Dr. Sasuke Nakao in Nepal in 1952. (d) Danjuro, one of the most popular cultivars. It was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

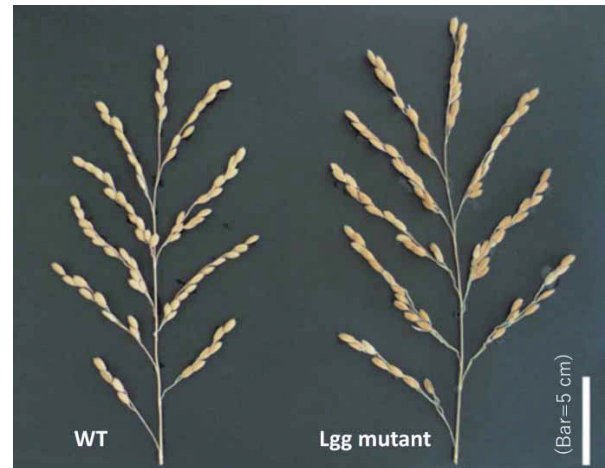


Figure 1. Phenotype of Large gain (*Lgg*). Harvested panicle and seeds.

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.

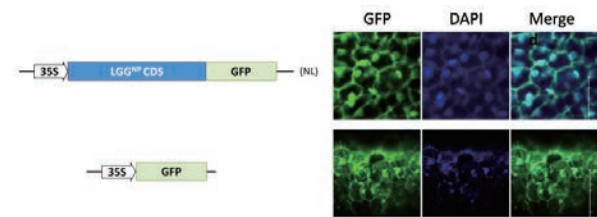


Figure 2. Subcellular localization of LGG. 35S:LGG^{NP}-GFP is expressed in rice calli. 4',6-Diamidino-2-phenylindole is used for nuclear staining. Bar = 20 μm.

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

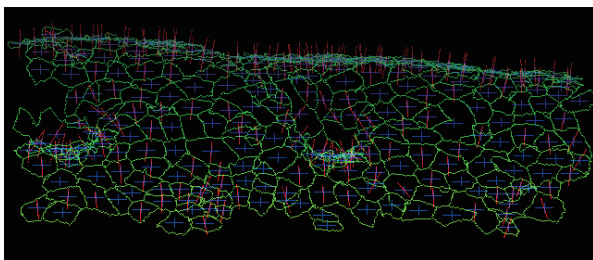


Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

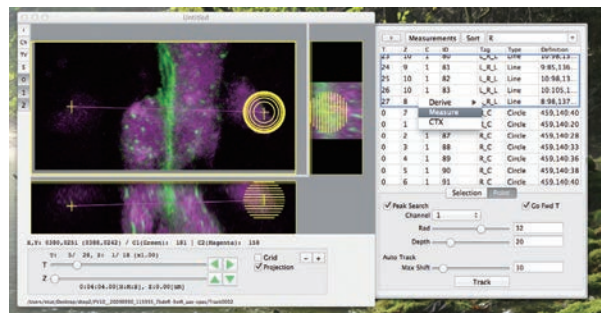


Figure 2. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

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., 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
- 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

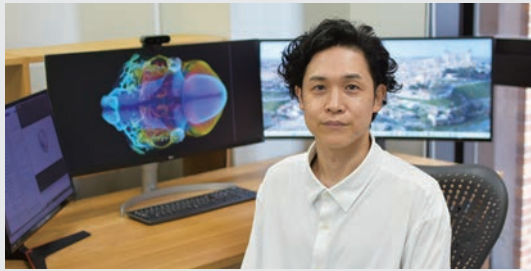


Image analysis is an important element in the understanding of life science. It makes the quantification of phenomena through the extraction of meaningful information from a large amount of images, and the appropriate expression of those information, possible. To this end, I have been developing image 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-

Simultaneous multifunctional analysis

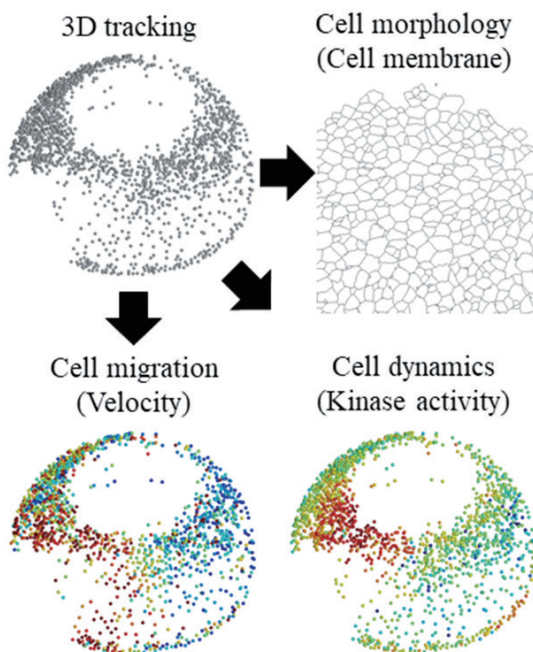


Figure 1. Simultaneous multifunctional analysis of early embryonic development

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.

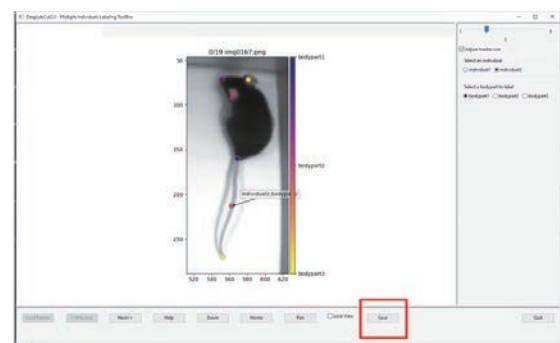



Figure 2. A website that explains how to use image analysis software

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.

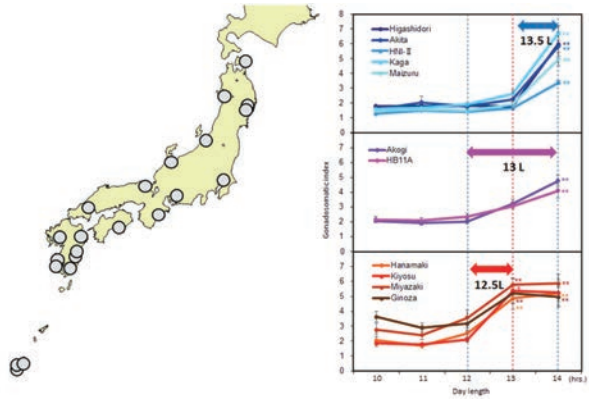


Figure 1. Differences in critical day length (right) were detected among medaka populations that had originated in various latitudes (left).

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 F₂ 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).

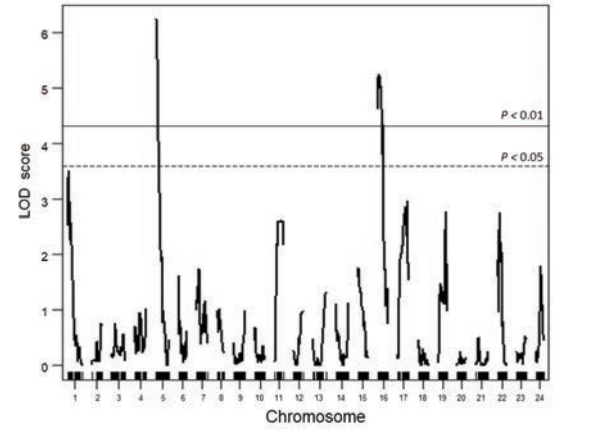


Figure 2. Result of QTL analysis for critical day length.

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., *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.

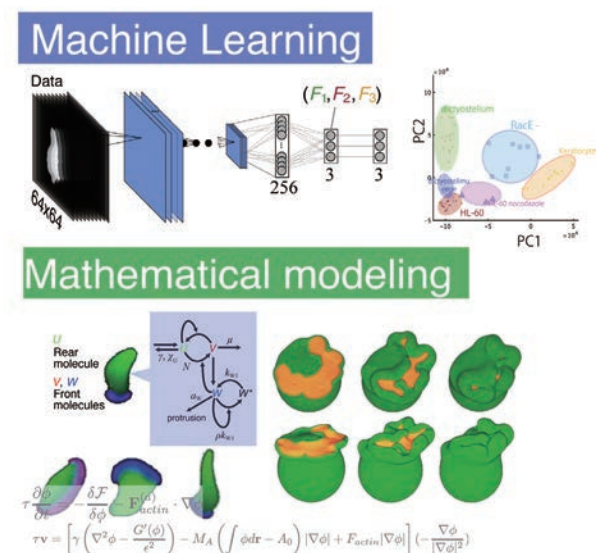


Figure 1. Researches combining machine learning and mathematical modeling.

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 LABORATORY

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

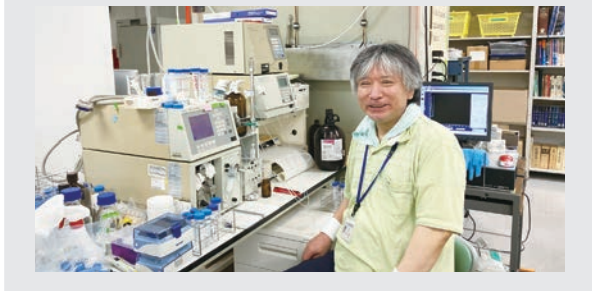


The first Open Laboratory in operation

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

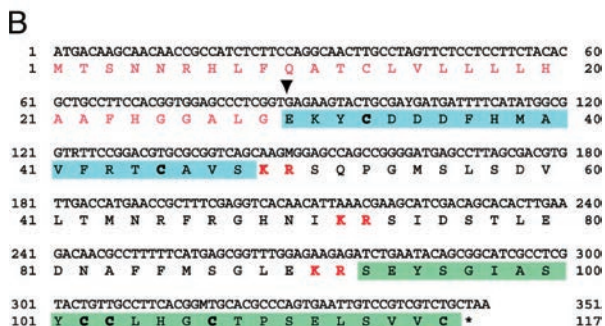


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

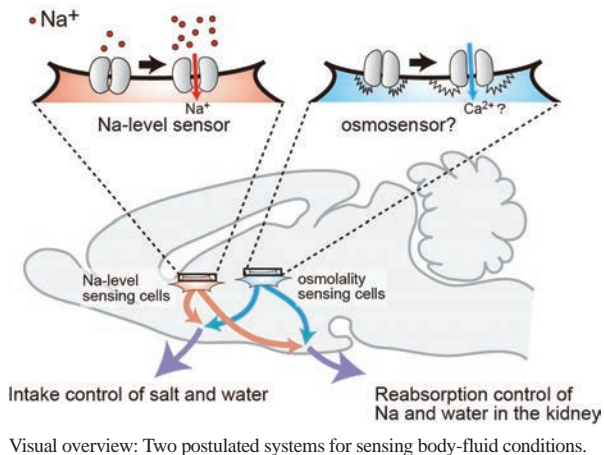
Sequence analysis by capillary sequencer was carried out from the tilling library of *O. latipes* in order to analyze the function of fish female reproductive hormone E2 as a part of our collaborative research. Upon the discovery of aromatase mutants, a detailed analysis was carried out using these strains.

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 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 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 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. Na_x is preferentially expressed in specific glial cells of sensory circumventricular organs (sCVOs) including the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT). We have found that Na_x signals in these brain regions deficient in a blood-brain barrier are involved in the control of salt intake.

We recently demonstrated that Na_x signals are also involved in the control of water intake behavior. 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, 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.

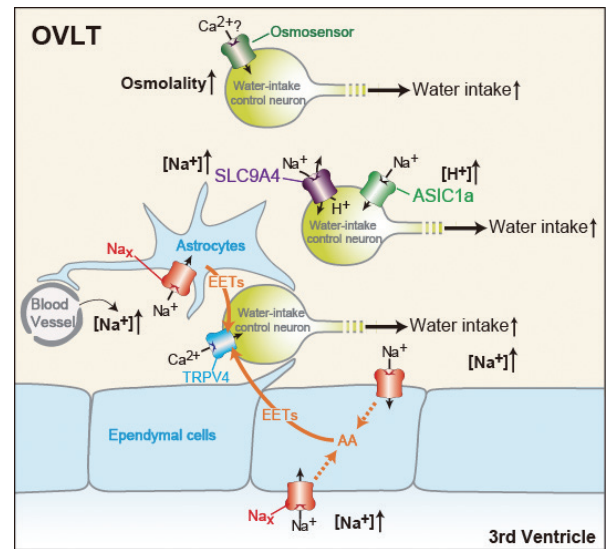


Figure 1. Schematic drawings of sensing mechanisms of body-fluid conditions in the OVLT responsible for the induction of water intake. AA, arachidonic acid.

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 $[\text{Na}^+]$ 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 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 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 Na_x -KO mice. Thus, water intake induced by $[\text{Na}^+]$ increases in body fluids may be explained by the 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:

[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⁺] 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_x-positive glial cells in the organum vasculosum laminae terminalis produce epoxyeicosatrienoic acids to induce water intake in response to increases in [Na⁺] in body fluids. *Neurosci. Res.* 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.

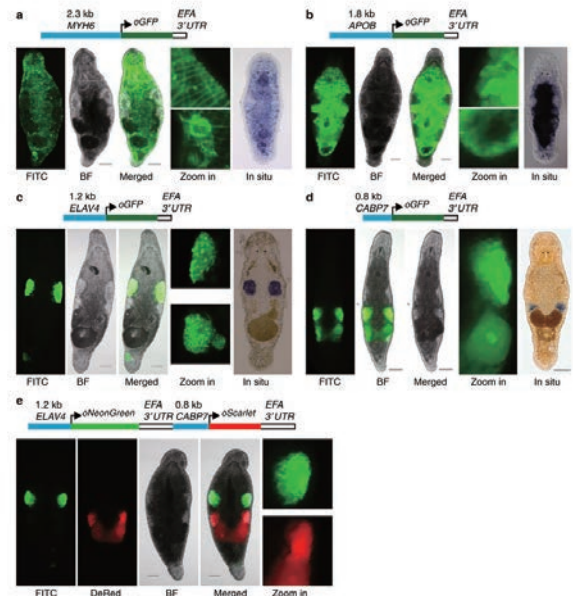


Figure 1. Tissue specific transgenic line of *Macrostomum lignano*.

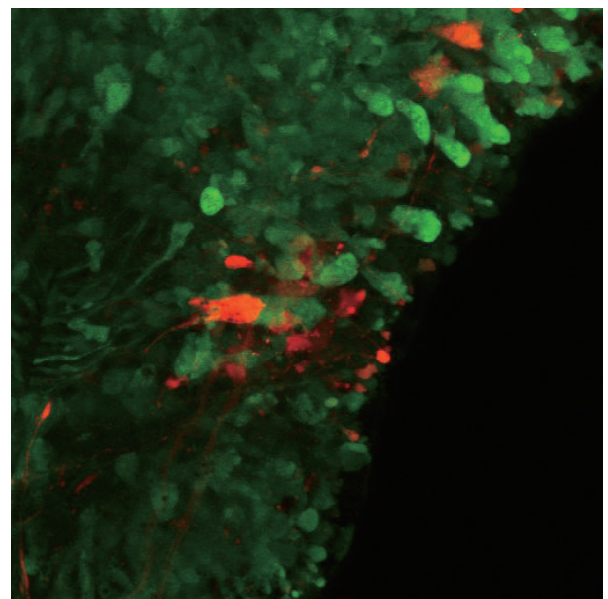
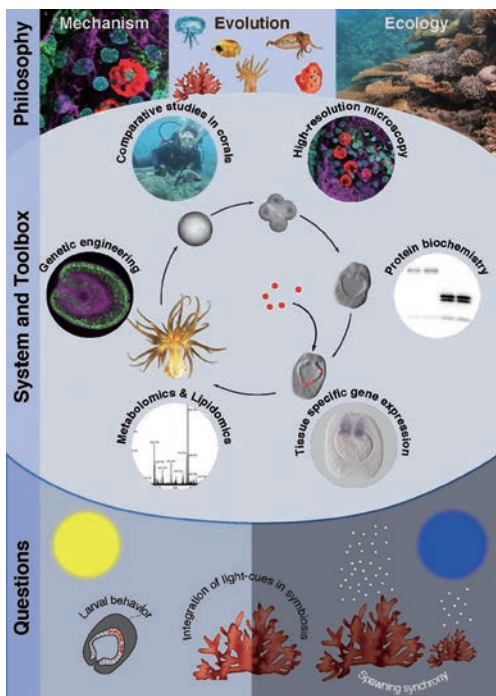


Figure 2. Expression of mScarlet under the HSP20 promoter 24 hours after induction using IR-LEGO

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.

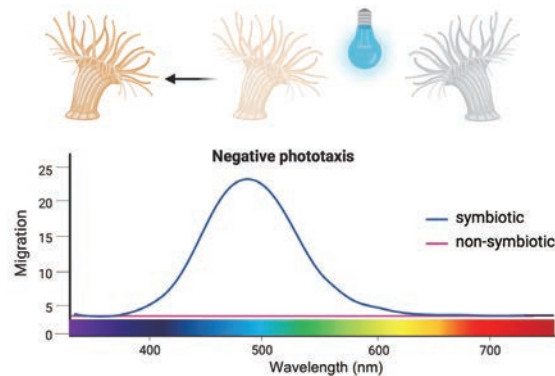


Figure 1. Wavelength specificity of negative phototaxis in symbiotic *Aiptasia*.

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*.

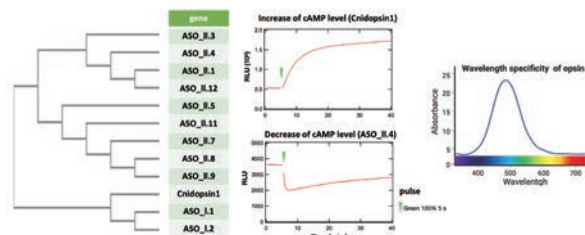


Figure 2. Functional analysis of *Aiptasia* opsins cloned in this study.

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.

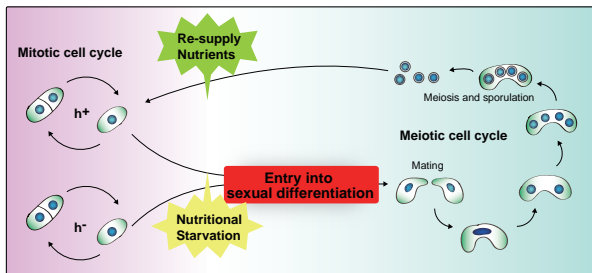


Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting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

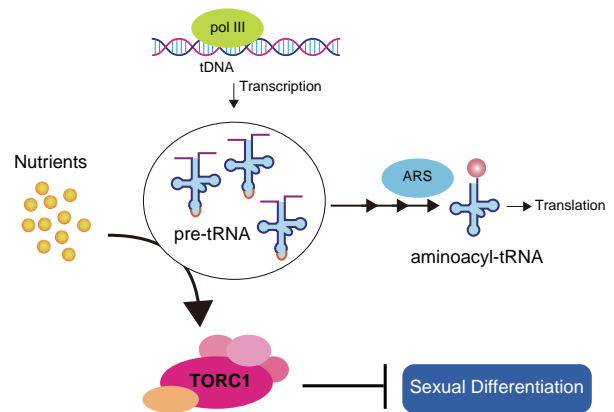


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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.

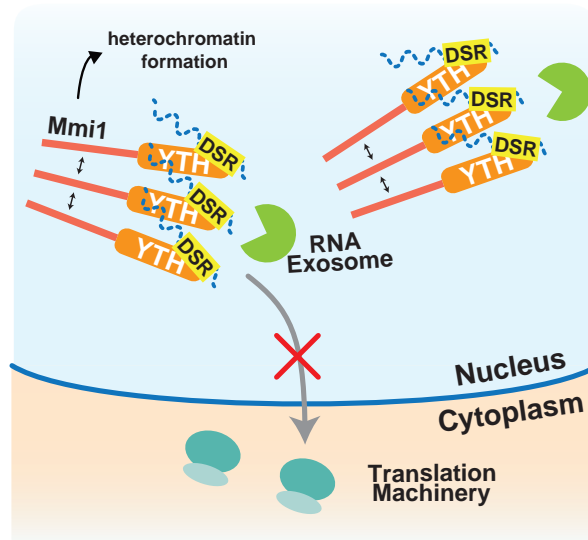


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

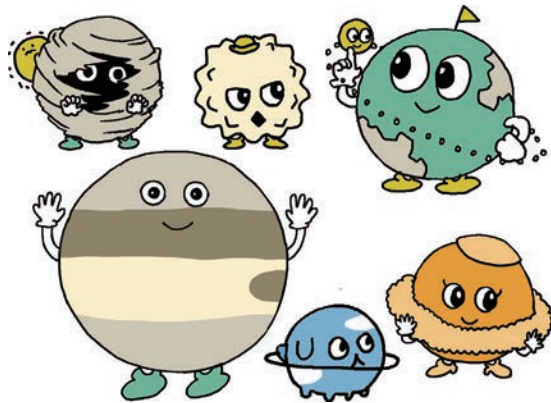


Figure 1. ABC mascot characters: “Exoplanet Friends” (Credit: Hayanon Science Manga Studio)

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.

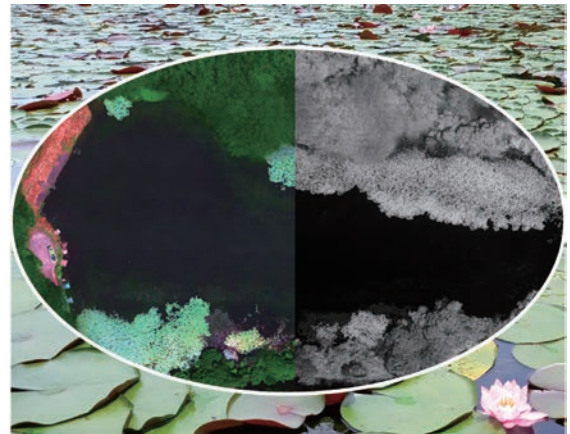


Figure 2. Remote sensing of light reflectance of floating leaf of waterlily. Light reflectance in visible light (BGR image on the left) and NIR (gray scale image on the right) were obtained by the drone-based multiband sensor.

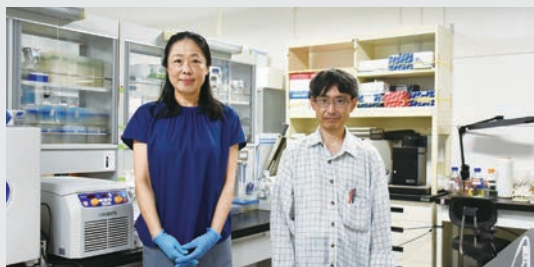
NIR driven photosynthesis around red dwarfs

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.

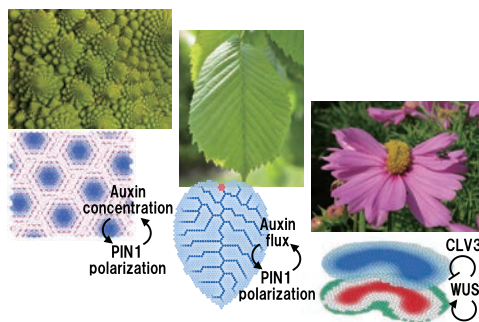


Figure 1. (Left) Phyllotaxis pattern is self-organized by the feedback regulation between auxin concentration and PIN1 polarization. (Middle) Leaf venation pattern is self-organized by the feedback regulation between auxin flux and PIN1 polarization. (Right) Shoot apical meristem (SAM) is self-organized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

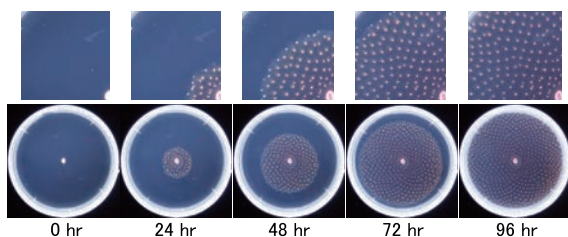


Figure 2. An example of self-organized colony patterns induced by chemotaxis in *E. coli* population.

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.

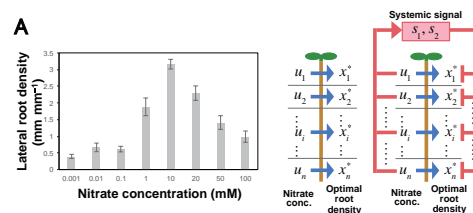


Figure 3. (A) Root growth in the homogeneous availability of nitrogen nutrition. (B) Schematic representation of the spatial regulation of the optimal resource allocation in response to nutrient availability. (right) In the low nutrient availability, the optimal root density is promoted by nutrient through 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:

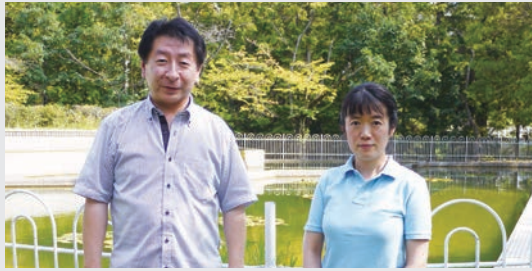
[Original paper]

- 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

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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.

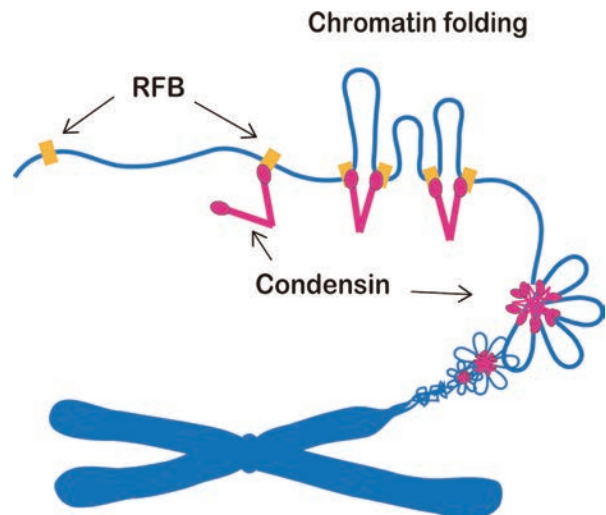


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.