LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Amino acids are the most important nutrients used in protein building; therefore, their perception is essential for all cells' existence. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. On one hand, TORC1 is involved in amino acid sensing, regulation of protein synthesis, the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. So far, it is not clear whether TORC2 also perceives nutrient signals.

The aim of our research group is to reveal the molecular mechanisms of how TORC1 receives amino acid signals and how the TORC1/2 pathways regulate each phenomenon. We have been studying Tor signaling in the budding yeast *Saccharomyces cerevisiae*, and have found three novel TOR signaling pathway branches (Figure 1). Recently, we found that tRNA plays a pivotal role in the ability of TORC1 to sense amino acids (Figure 1).

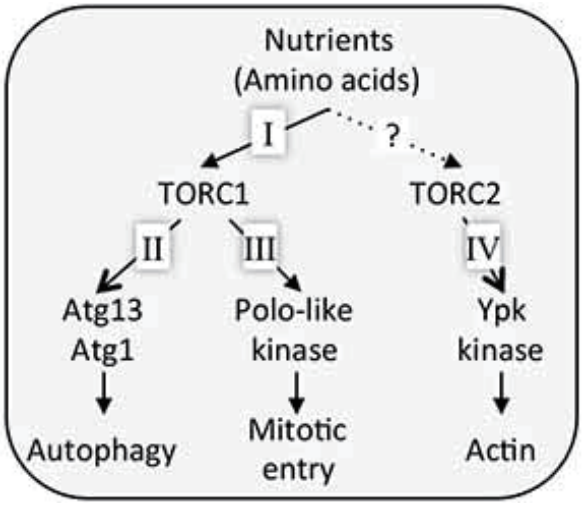

Figure 1. Tor signaling pathway for budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

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

We conducted genetic research and discovered the involvement of (aminoacyl-) tRNA in TORC1 regulation. Biochemical *in vitro* TORC1 assay also revealed that tRNA directly inhibits TORC1 kinase activity. Reducing cellular tRNA molecules desensitizes TORC1 inactivation by nitrogen starvation *in vivo*. Based on these results, a TORC1 regulatory model was proposed that contends that free tRNA released from protein synthesis under amino acid starvation inhibits TORC1 activity. Therefore, TORC1 employs a tRNA-mediated mechanism to monitor intracellular amino acids (Figure 2).

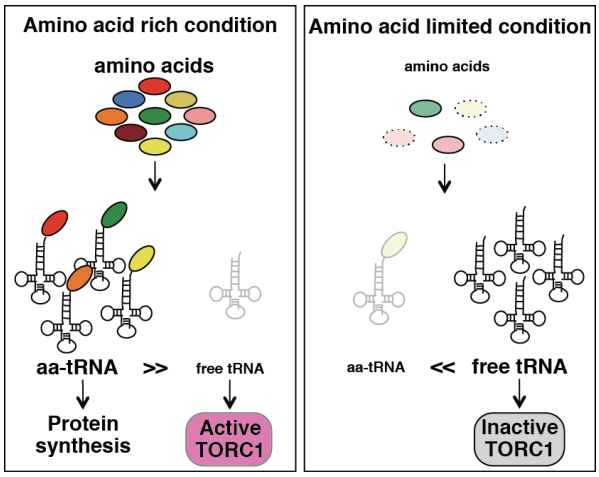

Figure 2. A schematic model of how amino acid is sensed by TORC1. Cytosolic free tRNA inactivates TORC1 under amino acid limited condition.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

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

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

Publication List:

〔Original Paper〕

● Baba, M., Tomonaga, S., Suzuki, M., Gen, M., Takeda, E., Matsuura, A., Kamada, Y., and Baba, N. (2019). A nuclear membrane-derived structure associated with Atg8 is involved in the sequestration of selective cargo, the Cvt complex, during autophagosome formation in yeast. Autophagy *15*, 423-437. doi: 10.1080/15548627.2018.1525475

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

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

I. Gonadotropins in the starfish, *Patiria pectinifera*

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

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

II. Search for reproductive hormones in echinoderms

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

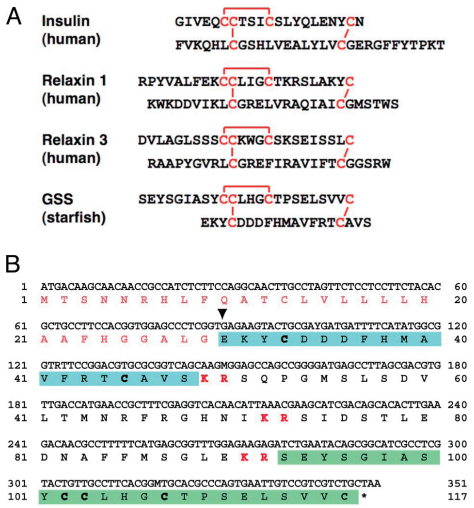

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

them, named cubifrin, which is an IWMGY-amide peptide, is in the sea cucumber *Aposticopus japonicus*. The others are in preparation for publication.

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. In particular, relaxin like peptide precursor genes and insulin/IGF like peptide precursor genes were identified from many species. We are producing these neuropeptides by biological methods (*e.g.* bacterial systems and yeast systems) to provide to collaborating researchers for biological assays.

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

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

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

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

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

I. Flower pigmentation patterns

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

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

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

II. Recreating the lost morning glory

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

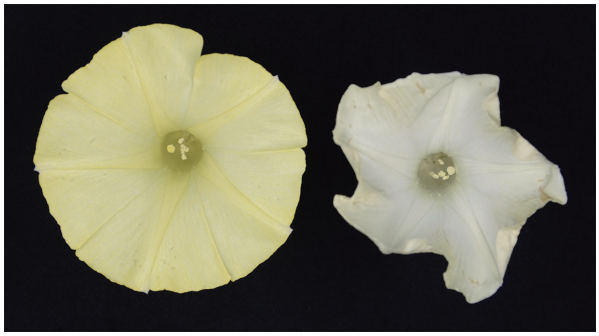

Figure 2. Flowers of the recreated 'phantom morning glory' (left), and the host plant (right). The host plant often exhibits shriveled flowers with necrotic cells, the transgenic plants produced fully opened flowers with few necrotic cells.

III. BioResource of morning glories

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glories. In this project, we are collecting, maintaining and distributing standard and mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* is one of the most popular floricultural plants in Japan, and has a 100-year history of extensive genetic studies related to it. Our collection includes 220 lines and 177,000 DNA clones. The whole genome sequence, the transcriptome sequences, as well as the end sequences of the DNA clones can be viewed via the *I. nil* genome database (http://viewer.shigen.info/asagao/index.php).

Publication List:

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

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*

Although transposons occupying a large portion of the genome in various plants were once thought to be junk DNA, they play an important role in genome reorganization and evolution. Active DNA transposons are important tools for gene functional analysis. The endogenous non-autonomous transposon, nDart1, in rice (Oryza sativa L.) is said to generate various transposon-insertion mutants because nDart1 elements tend to insert into genic regions under natural growth conditions. The transpositions of nDart1 were promoted by an active autonomous element, aDart1-27, on chromosome 6. By using the endogenous nDart1/aDart1-27 system in rice, a large-scale nDart-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation. The nDart1/aDart1-27 system was introduced into a rice variety, Koshihikari, named MK-1. 3000 MK-1 plants were grown in field conditions (IPSR, Okayama Univ.). All plants' genomes were isolated for identifying the insertion sites of nDart1.

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

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

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

II. Analysis of *Lgg* mutants

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

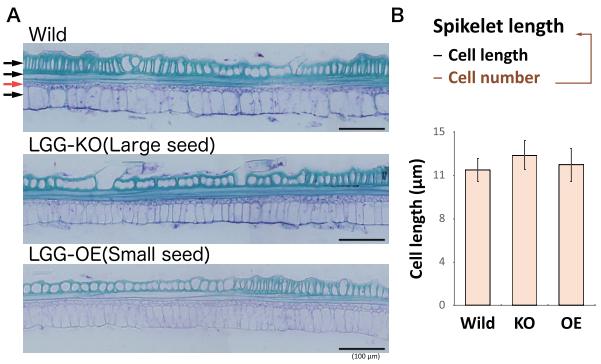


Figure 2. The section and cell number of lemma in transgenic plants. Bar = 100 μm. (A) the section of lemma in rice plants. The red arrow indicates counted cells. (B) Graph of cell number of lemma

Publication List:

〔Original papers〕

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

LABORATORY OF BIOLOGICAL DIVERSITY

SAKUTA Group

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

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

I. Thirst control by Na_x and TRPV4

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

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

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

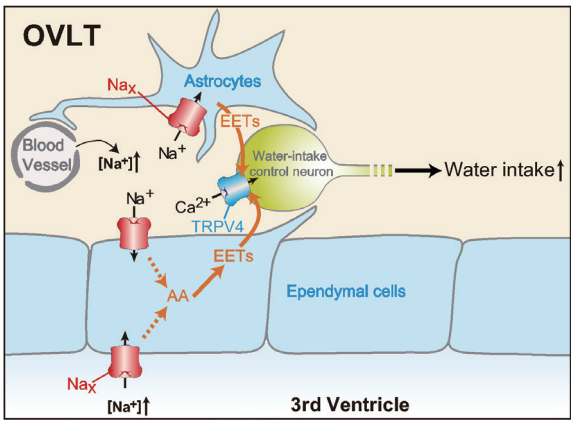


Figure 1. Proposed mechanisms for water intake induction by increases in $[Na^+]$ in body fluids via Na_x activation in the OVLT. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

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

Publication List:

〔Original papers〕

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

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

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

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

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

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

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

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

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in developing embryos is considered to be an important model for collective cell migrations. Drastic cell rearrangements lead to drastic structural changes in building elaborate organs such as the tubular network of *Drosophila* trachea. We are developing a software pipeline, which automatically recognizes individual cell shapes out of 3D space and tracks them through time. This system extracts cell boundaries and reconstitutes cell shapes as a skeletonized chain of voxels spanning 3D space. This abstract form of cell visualization makes it possible to describe morphometric quantities and kinetics of cells in single-cell resolution (Figure 1). These morphometric quantities allow us to perform comparative studies on shapes and behaviors more precisely among several experimental conditions, to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system (Shinoda *et al*.).

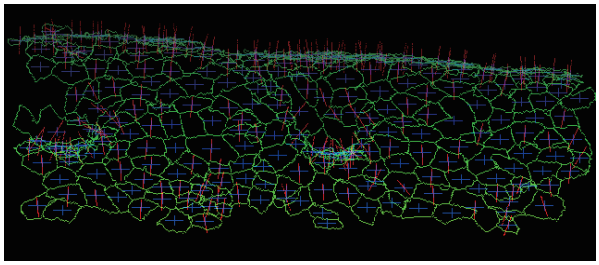


Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

II. Image processing pipeline for 3D cell culture

To elucidate the relationship between mechanical forces and epithelial deformation, we developed an image processing pipeline for segmentation of nucleus within 3D culture of MDCK cells. This pipeline automated a segmentation/quantification process of a large number of images acquired by several experimental conditions for subsequent statistical analysis (Nishimura *et al*.).

III. Software for manual image quantification

Biologically significant imaging features are not always significant to computational algorithms due to their structural instability. This kind of difficulty requires human eye inspection for feature extractions. A GUI (Graphical User Interface) application we have developed can easily visualize 4D imaging data and has made manual feature annotations easier (Figure 2). This application is freely available at our website (https://bioimageanalysis.jp/).

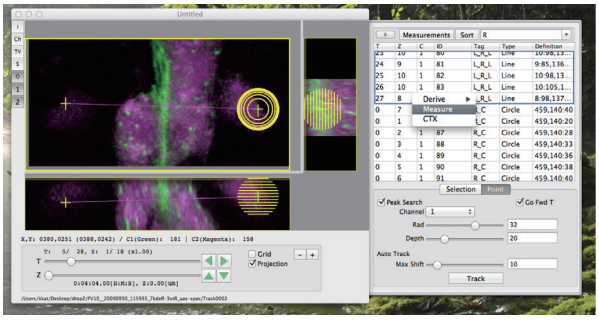


Figure 2. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

Publication List:

〔Original papers〕

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

LABORATORY OF BIOLOGICAL DIVERSITY†

OHTA Group

Specially Appointed Assistant Professor:
OHTA, Yusaku

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

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

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

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

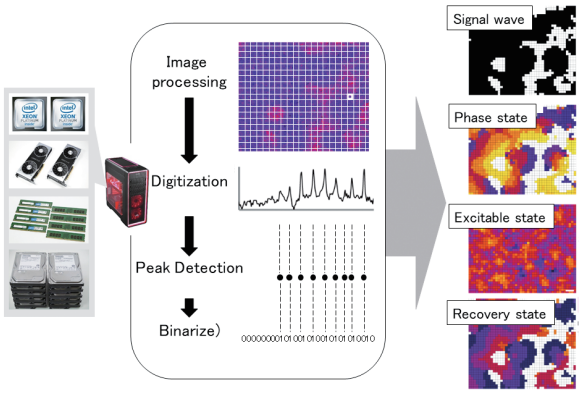


Figure 1. Example of automated image analysis

II. Research support by image analysis

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

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

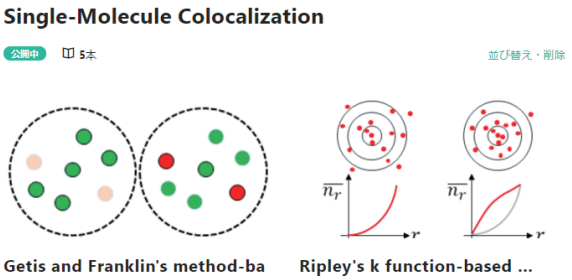


Figure 2. A website with topics related to image analysis

Publication List:

〔Original paper〕

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

LABORATORY OF BIOLOGICAL DIVERSITY†

SHINOMIYA Group

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

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

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

I. Underlying mechanisms that define critical day length and temperature

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

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

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

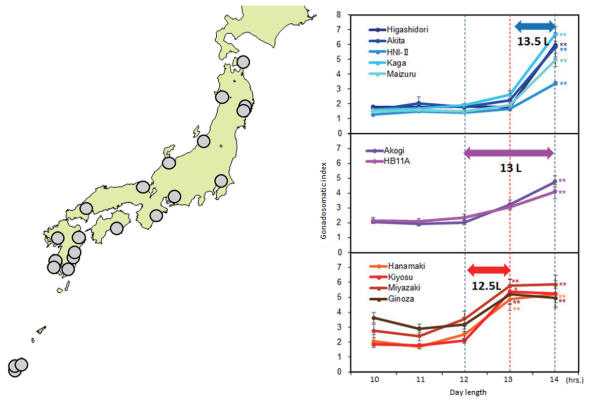


Figure 1. Medaka populations used in our study (left). Differences of critical day length among medaka populations (right).

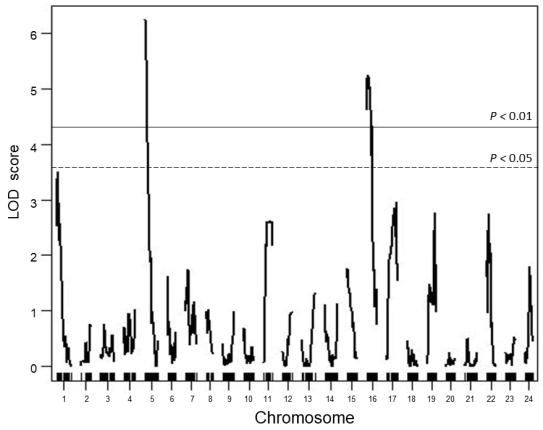


Figure 2. Result of QTL analysis for critical day length.

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

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

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

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

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

Publication List:

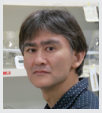
〔Original papers〕

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

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

LABORATORY OF BIOLOGICAL DIVERSITY

YAMASHITA AND OTSUBO Group



Specially Appointed Associate Professor
YAMASHITA, Akira

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

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

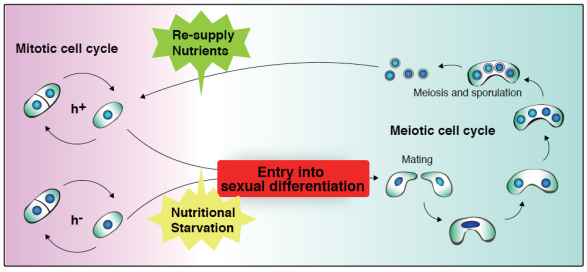


Figure 1. The life cycle of the fission yeast *S. pombe*. *S. pombe* cells mitotically proliferate under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. The resulting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

I. Signaling pathways that regulate the onset of sexual differentiation

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

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich mediums in restrictive temperatures. To gain an insight into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions, as *tor2* mutants do. We designated these mutants as *hmt*, which stands for hypermating and temperature-sensitive growth. We cloned the responsible genes and found that five of the eight responsible genes encoded tRNA-related factors. The *hmt1* and *hmt2* genes encoded aminoacyl-tRNA synthetases for asparagine and proline, respectively. The *hmt3* gene encodes tRNA adenosine-34 deaminase. The *hmt4* is identical to *rpc34*, which encodes a subunit of RNA polymerase III. The *hmt5* is identical to *sfc4*, which encodes a subunit of the RNA polymerase III-specific general transcription factor IIIC. In the *hmt1-5* mutants, TORC1 activity is downregulated, suggesting that the products of these *hmt* genes may function upstream of TORC1. We also found that the expression of tRNA precursors decreases upon nitrogen starvation occurring. Furthermore, overexpression of tRNA precursors prevents TORC1 downregulation in response to nitrogen starvation and represses the initiation of sexual differentiation. Based on these observations, we have proposed that tRNA precursors act as key signaling molecules in the TORC1 pathway in response to nitrogen availability (Figure 2).

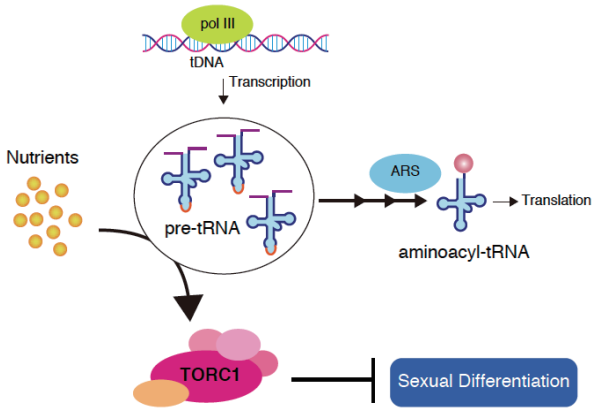


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

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

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

A YTH-family RNA-binding protein Mmi1 plays a crucial role in the selective elimination system of meiosis-specific transcripts during the mitotic cell cycle. Mmi1 recognizes a region termed DSR (Determinant of Selective Removal) in meiotic transcripts, which is enriched by repeated hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machin-

ery. Mmi1 also induces the formation of facultative heterochromatin at a subset of its target genes. Furthermore, Mmi1 regulates the termination of transcription of its target genes. Mmi1-mediated termination of an upstream non-coding RNA ensures the expression of downstream genes, one of which encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) essential for the initiation of sexual differentiation.

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

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

III. Cellular responses to atmospheric pressure plasma

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

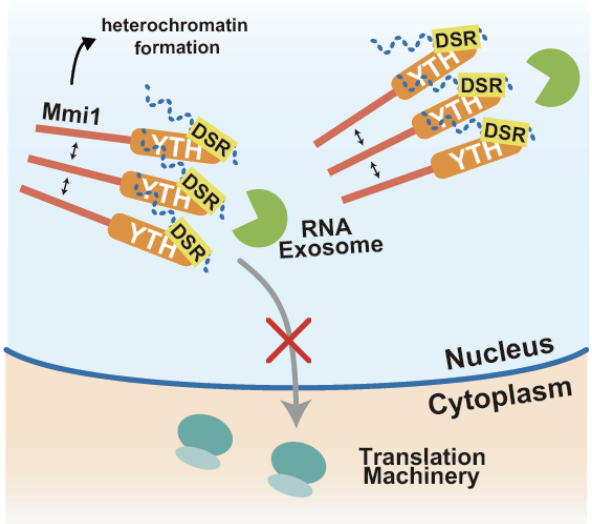


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear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

Publication List:

〔Original papers〕

- Lee, S.Y., Hung, S., Esnault, C., Pathak, R., Johnson, K.R., Bankole, O., Yamashita, A., Zhang, H., and Levin, H.L. (2020). Dense transposon integration reveals essential cleavage and polyadenylation factors promote heterochromatin formation. Cell Rep. *30*, 2686-2698. doi: 10.1016/j.celrep.2020.01.094
- Shichino, Y., Otsubo, Y., Yamamoto, M., and Yamashita, A. (2020). Meiotic gene silencing complex MTREC/NURS recruits the nuclear exosome to YTH-RNA-binding protein Mmi1. PLoS Genet. *16*, e1008598. doi: 10.1371/journal.pgen.1008598
- Yoshimura, S., Aramaki, M., Otsubo, Y., Yamashita, A., and Koga, K. (2019). Controlling feeding gas temperature of plasma jet with Peltier device for experiments with fission yeast. Japn. J. Appl. Phys. *58*, SEEG03. doi: 10.7567/1347-4065/ab1473

〔Review article〕

- Yamashita, A. (2019). meiRNA, a polyvalent player in fission yeast meiosis. non-coding RNA *5*, 45. doi: 10.3390/ncrna5030045

NINS Astrobiology Center

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

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

Figure 1. Lyot 2019 Poster

Laboratory of Biological Diversity

TAKIZAWA Group

Specially Appointed Associate Professor
TAKIZAWA, Kenji

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

Remote sensing of vegetation red-edge

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

Floating plants in water world

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

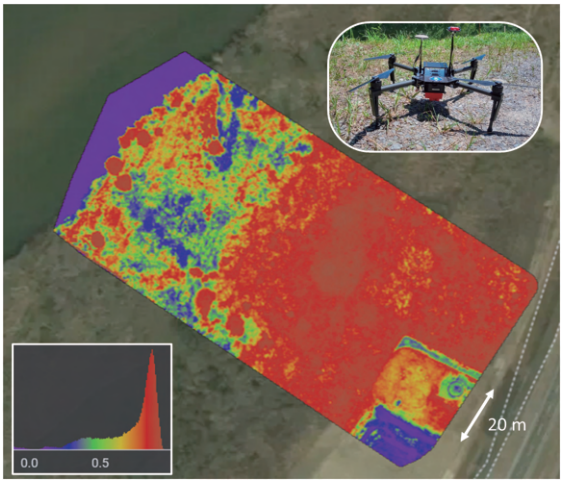


Figure 2. Normalized difference vegetation index (NDVI) survey for grassland on a riverbank. NDVI is a graphical indicator for green vegetation calculated from light reflectance in Red and NIR as (NIR-Red)/(NIR+Red). Grass covered area showed high NDVI (red color) in contrast with low NDVI (blue color)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

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

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

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

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

I. Dynamic relocalization of condensin during meiosis

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

II. Condensin-dependent chromatin folding

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

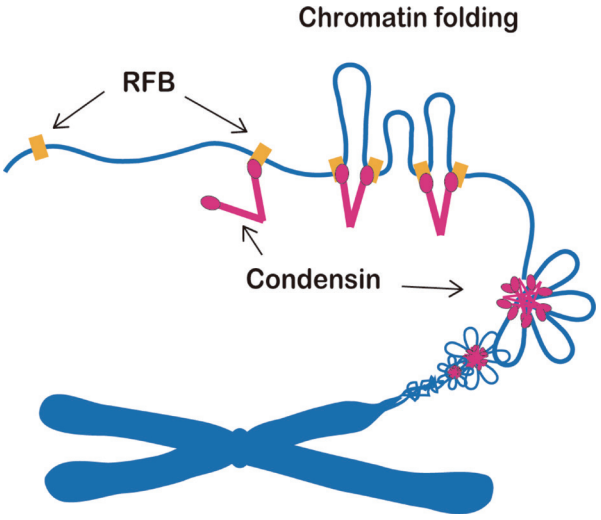


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

FUJITA Group

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

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

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

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

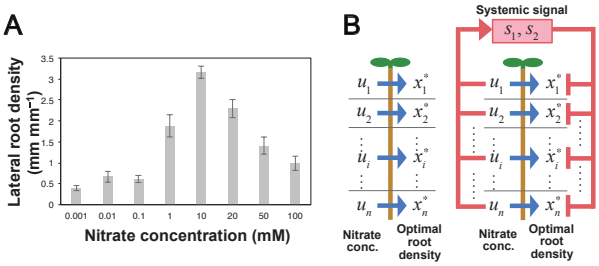


Figure 1. (A) Root growth in the homogeneous availability of nitrogen nutrition. (B) Schematic representation of the spatial regulation of the optimal resource allocation in response to nutrient availability. (left) In the low nutrient availability, the optimal root density is promoted by nutrient through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

II. Phyllotaxis pattern formation

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

Publication List:

〔Original papers〕

- Fujita, H., Hayashi-Tsugane, M., and Kawaguchi, M. (2020). Spatial regulation of resource allocation in response to nutritional availability. J. Theor. Biol. *486*, 110078. doi: 10.1016/j.jtbi.2019.110078
- Tokumoto, Y., Hashimoto, K., Soyano, T., Aoki, S., Iwasaki, W., Fukuhara, M., Nakagawa, T., Saeki, K., Yokoyama, J., Fujita, H., and Kawaguchi, M. (2020). Assessment of plant characteristics of *Polygala paniculata* (Polygalaceae) for evolutionary studies of legume-rhizobia symbiosis. J. Plant Res. *133*, 109-122. doi: 10.1007/s10265-019-01159-x
- Yonekura, T., Iwamoto, A., Fujita, H., and Sugiyama, M. (2019). Mathematical model studies of the comprehensive generation of major and minor phyllotactic patterns in plants with a predominant focus on orixate phyllotaxis. PLoS Comput. Biol. *15*, e1007044. doi: 10.1371/journal.pcbi.1007044