LABORATORY OF BIOLOGICAL DIVERSITY

MANO Group

Associate Professor: *MANO, Shoji*
Assistant Professor: *MANO, Shoji**
Postdoctoral Fellow: *KANAI, Masatake*
Visiting Scientist: *KAMIGAKI, Akane*
Technical Assistant: *HIKINO, Kazumi*
KATO, Kyoko
Secretary: *UEDA, Chizuru*

Plant cells can induce, degenerate and differentiate their organelles to adapt to environmental changes. This flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aims of our research group are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, and to understand the integrated functions of individual plants through organelle dynamics.

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

Peroxisomes are single-membrane bounded organelles, which are ubiquitously present in eukaryotic cells, and they are involved in various biological processes such as lipid metabolism and photorespiration. To understand peroxisome dynamics and functions, we have been analyzing a number of Arabidopsis mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants). Based on the analyses using these mutants a part of the mechanism of division, protein transport, degradation of peroxisomes, and the interactions of peroxisomes with other organelles were revealed (Figure 1). In addition, we found that peroxisomes are involved in the reproductive process. Therefore, peroxisome dynamics in gametes and gametophytes are currently under investigation.

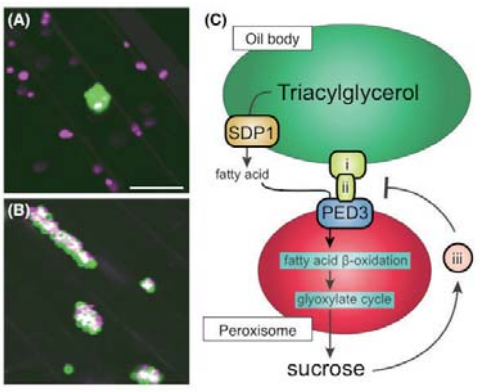

Figure 1. Physical interaction of peroxisomes and oil bodies. (A, B) Fluorescence observation of 3-day-old cotyledons of Arabidopsis wild-type (A) and *sdp1 (sucrose dependent 1)* plants (B). Magenta and green signals represent peroxisomes and oil bodies, respectively. Bar: 20 μm. (C) Model of the interaction of peroxisomes and oil bodies. PED3 (Peroxisome Defective 3) is the potential anchor protein on peroxisomal membranes. Sucrose acts as a negative signal for the interaction between both organelles.

II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves as foods and industrial materials. Storage reserves are different among different plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybean contains proteins as a major reserve. We are analyzing the mechanisms controlling oil and protein contents in seeds, and trying to apply our knowledge and techniques for increasing beneficial storage reserves.

III. Construction of The Plant Organelles Database 3 (PODB3)

PODB3 was built to promote a comprehensive understanding of organelle dynamics. PODB3 consists of six individual units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. Through these databases, users can obtain information on plant organelle responses to environmental stimuli of various tissues of several plant species, at different developmental stages. We expect that PODB3 will enhance the understanding of plant organelles among researchers.

Publication List:

〔Original papers〕

- Aboulela, M., Tanaka, Y., Nishimura, K., Mano, S., Kimura, T., and Nakagawa, T. (2017). A dual-site gateway cloning system for simultaneous cloning of two genes for plant transformation. Plasmid *92*, 1-11.
- Aboulela, M., Tanaka, Y., Nishimura, K., Mano, S., Nishimura, M., Ishiguro, S., Kimura, T., and Nakagawa, T. (2017). Development of an R4 dual-site (R4DS) gateway cloning system enabling the efficient simultaneous cloning of two desired sets of promoters and open reading frames in a binary vector for plant research. PLoS ONE *12*, e0177889.
- Hayashi, M., Tanaka, M. Yamamoto, S., Nakagawa, T., Kanai, M., Anegawa, A., Ohnishi, M., Mimura, T., and Nishimura, M. (2017). Plastidial folate prevents starch biosynthesis triggered by sugar influx into non-photosynthetic plastids of Arabidopsis. Plant Cell Physiol. *58*, 1328-1338.
- Kanai, M., Mano, S., and Nishimura, M. (2017). An efficient method for the isolation of highly purified RNA from seeds for use in quantitative transcriptome analysis. J. Vis. Exp. *119*, e55008.
- Watanabe, E., Mano, S., Yamada, K., Nishimura, M., Iuchi, S., Kobayashi, M., Uemura, M., and Kawamura, Y. (2017). Physiological analysis of Arabidopsis ecotype to investigate the freezing tolerance after cold acclimation process. Cryobiol. Cryotechnol. 63, 161-164.

〔Review article〕

- Watanabe, E., Mano, S., Hara-Nishimura, I., Nishimura, M., and Yamada, K. (2017). HSP90 stabilizes auxin receptor TIR1 and ensures plasticity of auxin responses. Plant Signal. Behav. *12*, e1311439.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Among various nutrients amino acids are the major nitrogen source; therefore, perception of the amino acid environment is essential for cells. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. TORC1 is involved in amino acid sensing, regulating 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 branches of the TOR signaling pathways (Figure 1).

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

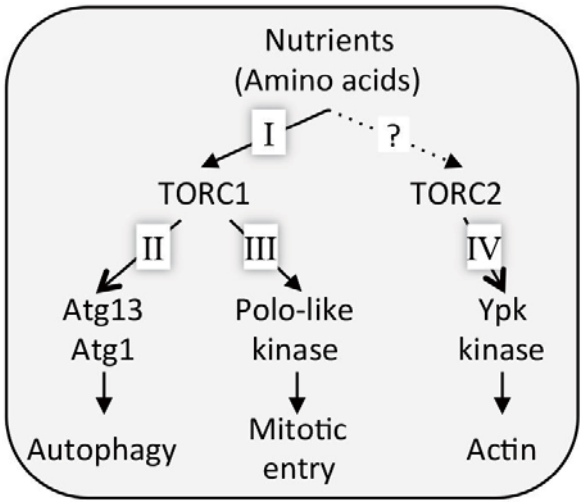

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

TORC1 is regulated by amino acids. Amino acids are fundamental nutrients, and 20 species of amino acids building proteins are not interchangeable with each other. Therefore, TORC1 should sense each amino acid individually. Mammalian mTORC1 is believed to be controlled by Rag GTPases and their regulators. However, Rag-factors are dispensable for amino acid sensing by TORC1 in the budding yeast, suggesting an alternative mechanism of TORC1 regulation.

Genetic investigation was done to discover 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 is proposed 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.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

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

We found 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.

III. TORC1 regulates mitotic entry via polo-like kinase

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at the G1 phase.

We demonstrated that TORC1 is also involved in another stage of the cell cycle, mitotic entry. Cdc5, the yeast polo-kinase mediates this regulation, and the nuclear localization of Cdc5 at G2/M transition is controlled by TORC1. In addition, we discovered a physiological role of TORC1 in mitosis; autophagy negatively controlled by TORC1 plays an important part in maintenance of genome stability under starvation conditions.

IV. Ypk kinase acts directly downstream of TORC2 to control actin organization

TORC2 has an essential function controlling polarity of the actin cytoskeleton. We found that Ypk2, a member of the AGC kinase family is directly phosphorylated by TORC2. The activated allele of *YPK2* can rescue a lethality caused by TORC2 dysfunction, suggesting that Ypk kinase is the major downstream protein of the TORC2 pathway.

Publication List:

〔Original paper〕

● Kamada, Y. (2017). Novel tRNA function in amino acid sensing of yeast Tor complex1. Genes Cells 22,135-147.

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, such as the egg development neurosecretory hormone of the mosquito, the egg-laying hormone of the sea hare, and the androgenic gland hormone of the terrestrial isopod, 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*, 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, acting 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 GSS referred to 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 of Kyushu Univ., we are searching for reproductive hormones in echinoderms; starfishes, brittle stars, sea urchins, sea cucumbers, and crinoids. The collaborators 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, an IWMGY-amide peptide, in the sea cucumber *Aposticopus japonicus*, the others are in preparation for publications.

We have identified many neuropeptides from our EST analysis of nerve tissues and many from RNA-seq and WGS data of the NCBI database. Especially 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, for providing to collaborators for biological assays.

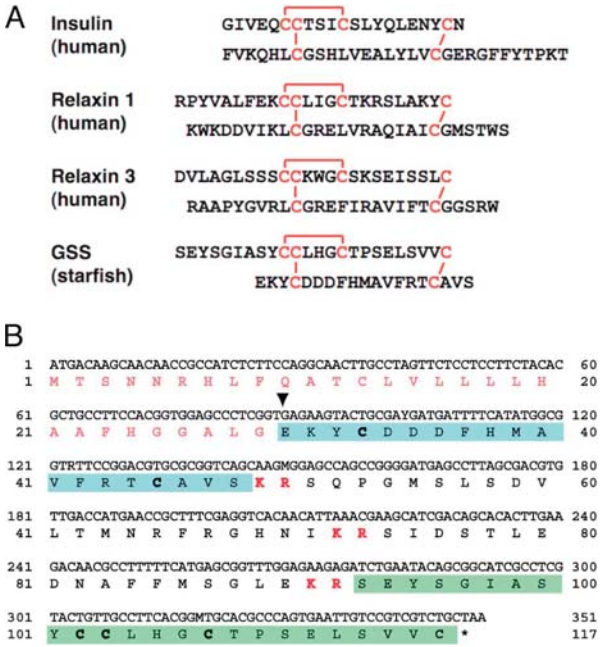

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

Publication List:

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

● Nakamoto, M., Shibata, Y., Ohno, K.. Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell Endocrinol. 2017 July 13.

LABORATORY OF BIOLOGICAL DIVERSITY

KOMINE Group

Assistant Professor: *KOMINE, Yuriko*

We have been interested in the developmental and evolutional aspects of the structure of mammalian brains. In a comprehensive analysis of homeobox genes expressed in the developing mouse neocortex, we isolated a novel gene *Zfhx2*, which encodes a transcription factor containing three homeobox domains and 18 Zn-finger motifs. *Zfhx2* is highly expressed in the developing mouse brain, particularly in differentiating neurons, and continues to be expressed throughout adulthood at a low level. Two other phylogenically related genes, *Zfhx3* and *Zfhx4*, have been identified. The former was reported to be expressed in a manner dependent on neural differentiation, and the latter is a candidate gene causing congenital bilateral isolated ptosis. Although these three genes are expressed in substantially similar patterns in the developing brain, common functional features have not been clarified. Currently we have been focusing on *Zfhx2* to reveal its function and mechanisms of expression control in the developing brain.

I. Expression of *Zfhx2* is negatively regulated by its own antisense RNA

We found that the antisense strand of *Zfhx2* is also expressed in the mouse brain in a manner complementary to the expression of *Zfhx2* mRNA (Figure 1). Although most neurons express *Zfhx2* mRNA immediately after their final mitosis, several types of neuron (e.g., granule cells in the olfactory bulb and pyramidal and granule cells in the hippocampus) express antisense RNA prior to *Zfhx2* mRNA during the early phase of their differentiation. By generating a gene-targeting mouse line in which *Zfhx2* sense RNA is expressed but not antisense RNA, we showed that this antisense RNA has a negative regulatory role in the expression of *Zfhx2* mRNA. These observations suggest that the ZFHX2 protein might have a role in a particular step of neuronal differentiation, and in some types of neuron, this step might be delayed by the expression of antisense RNA.

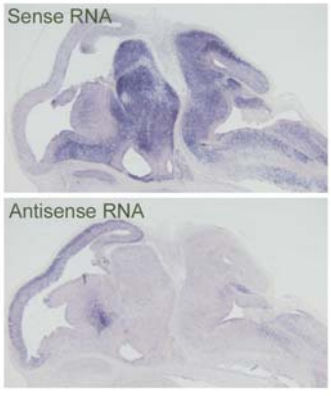

Figure 1. Expression of *Zfhx2* sense RNA (mRNA) and antisense RNA in the embryonic mouse brain. The antisense RNA was expressed where mRNA was not.

II. ZFHX2 might play roles in controlling emotional aspects

To elucidate the function of ZFHX2, we have also generated a *Zfhx2*-deficient mouse line. Although the production of the ZFHX2 protein is completely abolished in the homozygous mutant mice, the mice appear grossly normal and healthy. No anatomical abnormality has been observed in the mutant mouse brains so far examined. We hence subjected the *Zfhx2*-deficient mice to a comprehensive battery of behavioral tests to explore the physiological function of ZFHX2 in the nervous system. The homozygous *Zfhx2* deficient mice showed several behavioral abnormalities, namely, hyperactivity, enhanced depression-like behaviors, and an aberrantly altered anxiety-like phenotype. These behavioral phenotypes suggest that ZFHX2 might play roles in controlling emotional aspects through the function of monoaminergic neurons where ZFHX2 is expressed.

III. *ZFHX2* works also in pain perception process in human and mice

Recently, Cox's group in University College London found that a point mutation in the human *ZFHX2* gene segregates with the pain insensitivity found in a family with an inherited pain insensitive phenotype. Through the collaboration of several groups, including us, it has been shown that *ZFHX2* works as a critical gene for pain perception in humans and mice. Further work will resolve how the mutated *ZFHX2* gene contributes to the hypoalgesic phenotype and may help development of new analgesic drugs.

Publication List:

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

● Habib, A.M., Matsuyama, A., Okorokov, A.L., Santana, S., Bras, J.T., Aloisi, A.M., Emery, E.C., Bogdanov, Y., Follenfant, M., Gossage, S.J., Gras, M., Humphrey, J., Kolesnikov, A., Le Cann, K., Li, S., Minett, M., Pereira, V., Ponsolles, C., Sikandar, S., Torres, J.M., Yamaoka, K., Zhao, J., Komine, Y., Yamamori, T., Maniatis, N., Panov, K.I., Houlden, H., Ramirez, J.D., Bennett, D.L.H., Marsili, L., Bachiocco, V., Wood, J.N., and Cox, J.J. A novel human pain insensitivity disorder caused by a point mutation in *ZFHX2*. Brain 2017 Dec 14.

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 had a large impact on gene expression and evolution. To understand these genome dynamics in eukaryotes, especially in plants, we are characterizing 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, whereas a number of mutants displaying particular pigmentation patterns have been collected. Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate their flower pigmentation (Figure 1). We are currently characterizing detailed molecular mechanisms of these mutations.

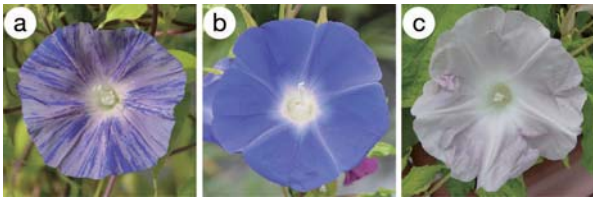

Figure 1. Flower phenotypes of the *duskish* mutant of *I. nil*. The phenotype is variable and displays variegated flowers (a), fully pigmented flowers (b), and pale grayish-purple flowers (c). As the flowers have the same genotype, epigenetic mechanisms are involved in the variable phenotypes.

II. Membrane transport of flower pigments

Anthocyanins are the most common flower pigments in Angiosperms including *I. nil*. They are synthesized in the cytosols and accumulate in the central vacuole in plant cells. Anthocyanin transport across the vacuolar membrane has long been debated. The transcriptional regulatory network of anthocyanin pigmentation supports involvement of an ATP binding caste (ABC) protein in the anthocyanin transport in *I. nil*. We started an international collaboration with researches in the Netherlands and Switzerland to reveal the function of the ABC protein.

III. Genome sequence information of the Japanese morning glory

In 2016, we reported an *I. nil* draft genome sequence with a scaffold N50 of 2.88 Mb, covering 98% of the 750 Mb genome. Scaffolds covering 91% of the genome sequence are anchored to 15 pseudo-chromosomes. A genome database for the genome sequence was built and opened to the public in 2017 (Figure 2). It includes a genome browser that enables users to analyze the 3,416 scaffolds (assembly name, Asagao_1.1), the 15 pseudo-chromosomes with the 3,095 scaffolds not anchored to the pseudo-chromosomes (Asagao_1.2), and the circular genomes of chloroplasts and mitochondria. The database provides BLAST, BLAT and keyword search services.

The genome sequence was also archived and used by several online databases and tools. The National Center for Biotechnology Information (NCBI) provides their own gene predictions (NCBI Ipomoea nil Annotation Release 100). The predicted genes are catalogued by the Kyoto Encyclopedia of Genes and Genomes (KEGG), and their functional information is visualized in the PATHWAY database that contains graphical representation of cellular processes (e.g. metabolism and signal transduction). Database Center for Life Science (DBCLS) equipped GGGenome and CRISPRdirect with the genome sequence. These tools facilitate ultrafast sequence search and rational design of CRISPR/Cas based genome editing target, respectively.

IV. BioResource of morning glories

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, 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. Our collections include 240 lines and 160,000 DNA clones. The end sequences of the DNA clones can be viewed from the *I. nil* genome database (Figure 2).

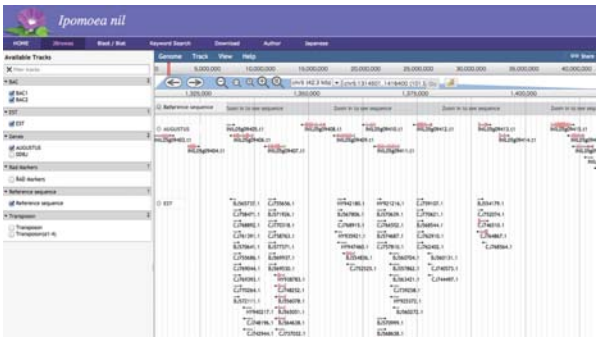

Figure 2. The *I. nil* genome database.
http://viewer.shigen.info/asagao/index.php

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, *nDart1-0*, in rice (*Oryza sativa* L.) is expected to generate various transposon-insertion mutants because *nDart1-0* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1-0* 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.). The genome of all plants were isolated for identifying insertion sites of *nDart1*.

I. Dominant mutation in rice

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

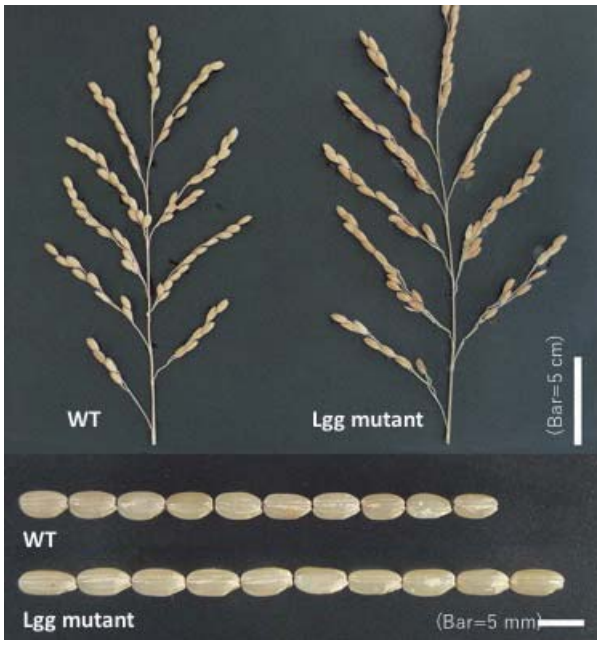

Figure 1. Phenotype of Large gain (Lgg). Harvested panicle and seeds.

II. Semidominant mutation in rice

The semidominant mutations produce the intermediate phenotype in individuals heterozygous for the gene concerned. The semidominant mutations were occasionally isolated from the MK-1, it was unclear what causes dominant mutations. Efficient selection and analysis of dominant mutants to analyze the gene functions in rice is very useful. Newly isolated, *Bushy dwarf tiller2* (*Bdt2*), which has the valuable agronomic traits of multiple tillering and dwarfism, was obtained from the MK-1 (Figure 2). Genetic analysis revealed the *Bdt2* mutation was controlled by two genetic elements. One *Bdt2* element, *Bdt2a* showed weak dwarf phenotype, another element, *Bdt2b* strengthened the effect of *Bdt2a*. A wild plant produces only 10 spikes or less, while *Bdt2a* produced 3 times as many, *Bdt2* produced 10 times the ears.

Figure 2. Phenotype of *Bushy dwarf tillers2* (*Bdt2*). (A) Two-month-old plants in the field (B) Segregants of *Bdt2* mutants. *Wild type* (left), *Bdt2a* (middle), *Bdt2*(right). (**C**) Abnormal bract of *Bdt2*.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: *JOHZUKA, Katsuki*
Technical Assistant: *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 shrinking the length of chromosome arms, 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, resulting in cell lethality. Chromosome condensation is mainly achieved by condensin, a hetero-pentameric protein complex, widely conserved from yeast to humans. Despite its conservation and importance for chromosome dynamics, how condensin works is not well understood. Recent studies reveal that condensin functions are not restricted to chromosome condensation and segregation during cell divisions. It is required for diverse DNA metabolism such as genome stability, transcriptional regulation, and cell differentiation.

Our research interest is to understand the mechanism and regulation of chromosome condensation. We have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. Microscopic observation 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 recruitment of condensin to the RFB site. These proteins are known as components of monopolin complex that are required for faithful segregation of homologous chromosomes during meiotic division I. During meiosis I, monopolin complex re-localizes from rDNA repeat to the centromere and acts for ensuring sister-chromatid co-orientation. Re-localization of Csm1/Lrs4 proteins suggested the re-localization of condensin from rDNA repeat to centromere. 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, is functioning as a cis-element for 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 on an ectopic chromosome arm with an interval of 15kb distance in the cell with 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 found 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 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 internal regions of 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.

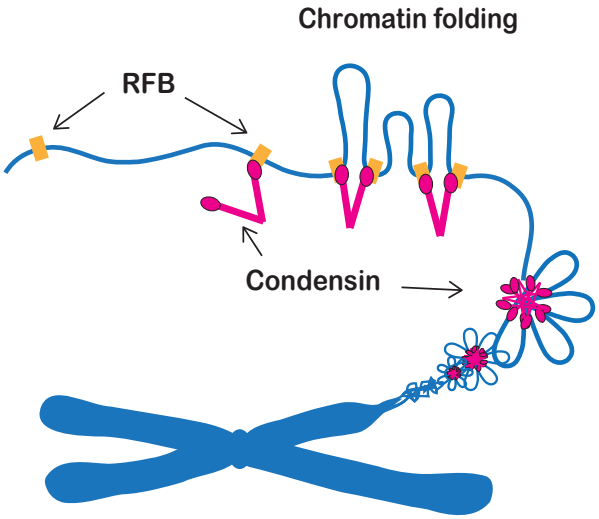

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

Specially Appointed Assistant Professor:
KATO, Kagayaki

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 an integrated result of individual cell behaviors in response to spatio-temporally controlled mechanisms.

To better understand the programs underlying organ formation, it is required to analyze individual cells' morphology and dynamics quantitatively. However, due to the massive images generated by 4D microscopy and their ambiguity, this made it difficult to perform these analyses.

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

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in the developing embryo is considered to be an essential model for collective cell migrations. Drastic cell rearrangements lead drastic structural changes to build 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 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.

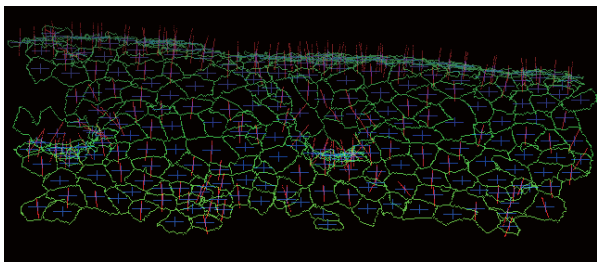

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

II. Local image feature tracking for tissue deformation analysis

Besides cell boundary extraction, we also developed a derived algorithm for particle image velocimetry (PIV). This system is designed to measure tissue deformation even though the imaging constraints do not allow identification of individual cells out of images. This implementation detects structural characteristics, such as uneven fluorescence distributed over the specimen and tracks these patterns along a time-series. Despite that the tissue was labeled with non-targeted cytoplasmic GFP, this tracking software successfully outlined developmental dynamics of *Xenopus* neuroectoderm (Figure 2).

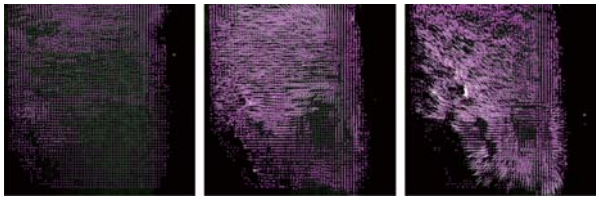

Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signals over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed the microscopy.

III. Software for manual image quantification

Biologically significant image 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 developed can easily visualize 4D imaging data and has made manual feature annotations easy (Figure 3). This application is freely available at our website (https://is.cnsi.nins.jp/).

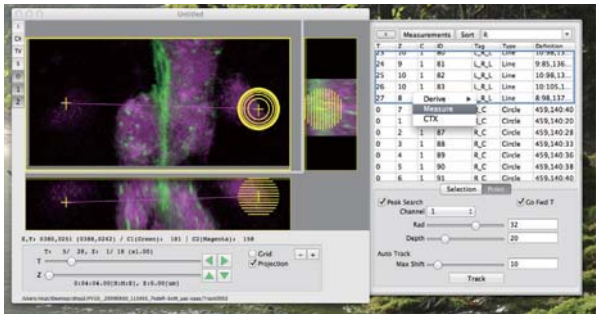

Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

LABORATORY OF BIOLOGICAL DIVERSITY

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Image processing methods significantly contribute to visualization of biomedical targets acquired from a variety of imaging techniques, including: wide-field optical and electron microscopy, X-ray computed tomography, magnetic resonance imaging and mammography. Quantitative interpretation of the deluge of complicated biomedical images, however, poses many research challenges. We have developed new computational methods based on mathematical morphology for quantitative image analysis. One of the most important purposes of image processing is to derive meaningful information, which is expressed as image structural properties. Mathematical morphology is a nonlinear image processing method based on set theory and is useful for the extraction of the structural properties from an image. It can be used as a fundamental tool to analyze biomedical images.

Novel contrast enhancement method based on mathematical morphology for medical diagnosis

Image processing is a crucial step in the analysis of medical imaging data. As such, it is fundamental to a wide range of biomedical imaging and clinical research fields. Image processing derives structural features, which are then numerically quantified by image analysis. Especially, in diagnostic imaging, contrast enhancement of structural details of lesion regions plays an important role. It improves image quality and supports clinician diagnosis. By using the contrast enhancement method, more accurate medical diagnosis can be expected. However, conventional image enhancement techniques also emphasize the noise and structure of various normal tissues other than lesion regions.

In this study, a contrast enhancement approach based on a new type of mathematical morphology is introduced. This method emphasizes only the structure of the lesion while suppressing the emphasis of noise and normal anatomical structure.

Mathematical morphology is a methodology for extracting shape and size information from an image. It involves configuration of a set of nonlinear operators that act on images by using structuring elements (SE). The SE, which indicates the shape characteristics in an image, is generally a small and simple binary image. The two basic morphological operators are dilation and erosion, from which many operations can be derived. However, since the size of lesions vary, in many cases it is not possible to process with fixed-size SE.

This proposed method is based on a morphological subtraction method. In this new type of morphological enhancement method, *h*-maxima transform is applied to the original medical image. The unwanted structures that surround the target are suppressed in the process of target enhancement. Furthermore, this new method has no restrictions on the size and shape of the target object that is to be enhanced.

This method was applied to enhancement of an abnormal region in the chest radiograph shown in Fig. 1. The arrow in Fig. 1(a) shows the position of the abnormality (lung nodule). Fig. 1(b) shows the image after contrast enhancement using the proposed method. From the result, the nodule region was clearly distinguished from the surrounding tissues.

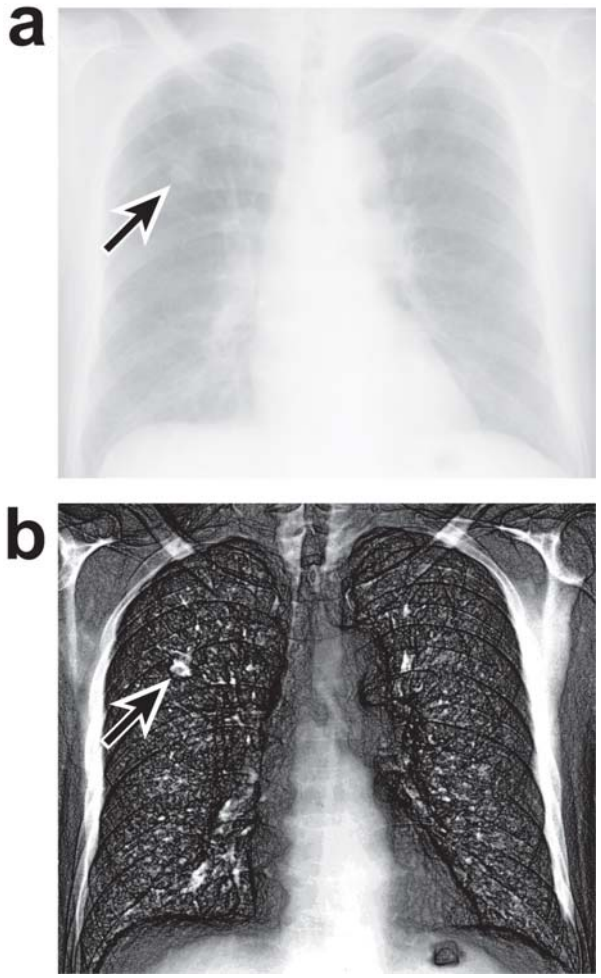

Figure 1. Contrast enhancement result for chest x-ray image: (a) Original chest radiograph. (b) Contrast enhanced image by using the proposed method. The image was obtained from a standard digital image database of chest lung nodules and non-nodules (Shiraishi et al., *ARJ Am. J. Roentgenol.* 174, 2000).

Publication List:

〔Original paper〕

● Osanai, Y., Shimizu, T., Mori, T., Yoshimura, Y., Hatanaka, N., Nambu, A., Kimori, Y., Koyama, S., Kobayashi, K., and Ikenaka, K. (2017). Rabies virus-mediated oligodendrocyte labeling reveals a single oligodendrocyte myelinates axons from distinct brain regions. Glia *65*, 93-105.