LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Thus, perception of the nutrient environment is also essential for cells. To recognize cellular nutrient conditions, eukaryotic cells employ Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as protein synthesis, cell cycle and autophagy. TORC1 is thought to act as a nutrient sensor, because rapamycin, a TORC1 inhibitor, mimics a starved condition. On the other hand, TORC2, whose function is insensitive to rapamycin, 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 Tor receives nutrient 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 pathway (Figure 1).

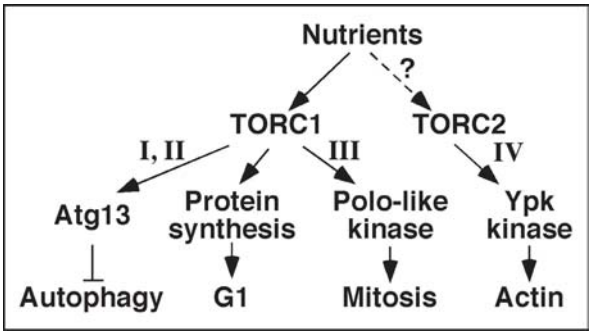

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. TORC1 phosphorylates Atg13, the molecular switch of autophagy

Autophagy is mainly a response to nutrient starvation, and TORC1 negatively regulates autophagy. The Atg1 kinase and its regulators, i.e. Atg13, Atg17, Atg29, and Atg31 collaboratively function in the initial step of autophagy induction, downstream of TORC1. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced following nutrient starvation or the addition of rapamycin. This regulation involves phosphorylation of Atg13.

We found that Atg13 is directly phosphorylated by TORC1. Phosphorylated Atg13 (during nutrient-rich conditions) loses its affinity to Atg1, resulting in repression of autophagy. On the other hand, under starvation conditions Atg13 is immediately dephosphorylated and binds to Atg1 to form Atg1 complex. Atg1 complex formation confers Atg1 activation and consequently induces autophagy. We determined phosphorylation sites of Atg13 and generated an unphosphorylatable Atg13 mutant (Atg13-8SA). Expression of Atg13-8SA induces autophagy bypassing inactivation of TORC1, the same as starvation treatment or rapamycin. These results demonstrate that Atg13 acts as a molecular switch for autophagy induction.

II. Monitoring in vivo activity of TORC1 by phosphorylation state of Atg13

Since Atg13 has turned out to be a substrate of TORC1, *in vivo* activity of TORC1 can be monitored by the phosphorylation state of Atg13. Various conditions and mutants have been examined to determine what kind of nutrients TORC1 recognizes and how nutrient signals are transmitted to TORC1.

III. How and why TORC1 regulates mitotic entry?

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at G1 (G0). Little is known, however, about whether or not TORC1 is involved in other stages of the cell cycle.

We generated a temperature-sensitive allele of *KOG1 (kog1-105)*, which encodes an essential component of TORC1, and found that TORC1 plays an important role in mitotic entry (G2/M transition). Since Cdc5, the yeast polo-kinase is mislocalized and inactivated in *kog1-105* mutant cells, TORC1 mediates G2/M transition via regulating polo-kinase. Recently 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. Ypk2 kinase acts at the downstream of TORC2 to control actin organization

Genetic studies have shown that TORC2 controls polarity of the actin cytoskeleton via the Rho1/Pkc1/MAPK cell integrity cascade. However, the target (substrate) of TORC1 was not yet identified. We found that Ypk2, an AGC-type of protein kinase whose overexpression can rescue lethality of TORC2 dysfunction, is directly phosphorylated by TORC2.

Publication List

〔Original paper〕

● Matsui, A., Kamada, Y., and Matsuura, A. (2013). The role of autophagy in genome stability through suppression of abnormal mitosis under starvation. PLoS Genetics *9*, e1003245.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

The aim of this laboratory is to research reproductive hormones in invertebrates 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, *Asterina 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, *Asterina 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/insulin-like peptides (Figure 1).

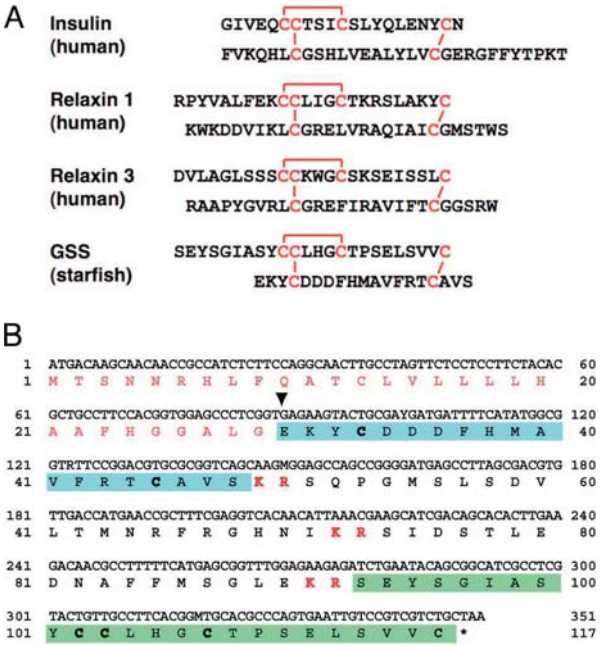

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

II. Search for reproductive hormones in invertebrates

In a collaborative effort with Prof. Yoshikuni's Laboratory of the Kyushu Univ. and Dr. Yamano and Dr. Awaji of the National Research Institute of Aquaculture, Fisheries Research Agency (NRIA), we are searching for reproductive hormones in invertebrates; sea urchin, sea cucumber, oyster, and shrimp. While the collaborators are partially purifying physiological materials which induce egg maturation from nerve extracts and analyzing them with a tandem mass spectrometer, we are creating and analyzing EST libraries from nerve tissues and developing a database of the mass analysis performed in this laboratory.

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

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

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 such genome dynamisms in eukaryotes, especially in plants, we are characterizing the flower pigmentation of morning glories.

I. Flower pigmentation patterns of the morning glories

Ipomoea nil (Japanese morning glory), *I. purpurea* (the common morning glory), and *I. tricolor* have been domesticated well as floricultural plants, and their various spontaneous mutations have been isolated. The wild type morning glories produce flowers with uniformly pigmented corolla, whereas a number of mutants displaying particular pigmentation patterns have been collected (Figure 1). Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

Margined, *Rayed* and *Blizzard* of *I. nil* are dominant mutations. While these mutants show distinct flower pigmentation patterns, the same pigmentation gene is repressed by non-coding small RNA in the whitish parts of the corolla. It is suggested that distinct regulation of small RNA cause the difference in pigmentation patterns. The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate flower pigmentation. We are currently characterizing detailed molecular mechanisms of these mutations.

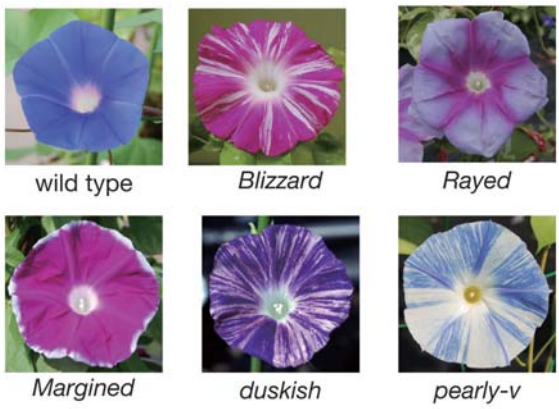

Figure 1. Flower phenotypes of the morning glories.

II. *de novo* sequencing of Japanese morning glory genome

Although morning glories are studied worldwide, especially in plant physiology and genetics, no whole nuclear genome sequences of any *Ipomoea* species are available. To facilitate the studies of our group as well as all morning glory researchers, we are conducting *de novo* genome sequencing of *I. nil*, having a genome of about 800 Mbp. We chose the Tokyo-kokei standard line for genome sequencing, and employed not only shotgun sequencing using high-throughput DNA sequencers but also BAC end sequencing. We are collaborating with several laboratories in Japan.

III. BioResource of morning glories

NIBB is the sub-center for 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* has been one of the most popular floricultural plants since the late Edo era in Japan. It has extensive history of genetic studies and also has many advantages as a model plant; simple genome, large number of mutant lines, and efficient self-pollination. Our collection includes 220 lines and 147,000 DNA clones.

IV. Flower color and vacuolar pH

Flower color is not only determined by pigments. It is dependent on several factors, such as colorless pigments, metal ions, and pH in the vacuole where flower pigments are accumulated. Petunia blooms red or violet flowers, and mutations in any one of the seven loci, named *PH1-PH7*, result in a bluish flower color (Figure 2). We successfully isolated *PH1* that encodes $P3_B$-ATPase, hitherto known as Mg^{2+} transporters in bacteria. Although PH1 itself is not a proton transporter, it can boost PH5 ($P3_A$-ATPase) proton transport activity that has been known to be essential for vacuolar hyperacidification. PH1 and PH5 physically interact with each other, and co-localize in the vacuolar membrane. The heteromeric P-ATPase pump of PH1 and PH5 is sufficient to hyperacidify vacuoles creating red pigmentation of petunia flowers.

Figure 2. The petunia unstable *ph1* mutant. The bluish pigmentation is due to a failure to hyperacidify vacuoles.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*
Visiting Scientist: *HAYASHI-TSUGANE, Mika*

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 could be easily generated under normal field conditions, and the resulting tagging lines were free of somaclonal variation.

I. A mutable albino allele in rice reveals that formation of thylakoid membranes requires *SNOW-WHITE LEAF1* gene Activation and Epigenetic Regulation of DNA Transposon *nDart1* in Rice

To understand chloroplast biogenesis and development, various chloroplast-defective mutants have been analyzed, yet they remain to be discovered. Although analyses of albino plants provide important information about mechanisms of plastid development, albino mutants are seedling lethal under natural growth conditions, owing to the complete loss of photosynthetic apparatus. Variegated mutants are excellent models for exploring the mechanism of chloroplast biogenesis because green and white sectors in the leaves allow an increased chance of survival. The variegation caused by somatic excision of DNA transposon is known as a mutable allele. The endogenous *nDart1*/*aDart1* tagging system is a powerful tool for investigating various unidentified and/or uncharacterized albino alleles. We report a novel variegated albino mutant, *snow-white leaf1-variegated* (*swl1-v*), caused by insertion and excision of *nDart1-0* in the *SNOW-WHITE LEAF1* (*SWL1*) gene (Figure 1). We have developed a specific method (nDart1-0-iPCR) for efficient detection of *nDart1-0* insertions and successfully identified the *snow-white leaf1* (*swl1*) gene in a variegated albino (*swl1-v*) mutant obtained from the *nDart1*-promoted rice tagging line. The variegated albino phenotype was caused by insertion and excision of *nDart1-0* in the 5'-untranslated region of the *SWL1* gene predicted to encode an unknown protein with the N-terminal chloroplast transit peptide. *SWL1* expression was detected in various rice tissues at different developmental stages. However, immunoblot analysis indicated that SWL1 protein accumulation was strictly regulated in a tissue-specific manner. In the *swl1* mutant, formations of grana and stroma thylakoids and prolamellar bodies were inhibited (Figure 2). This study revealed that SWL1 is essential for the beginning of thylakoid membrane organization during chloroplast development. Furthermore, we provide a developmental perspective on the *nDart1*-promoted tagging line to characterize unidentified gene functions in rice.

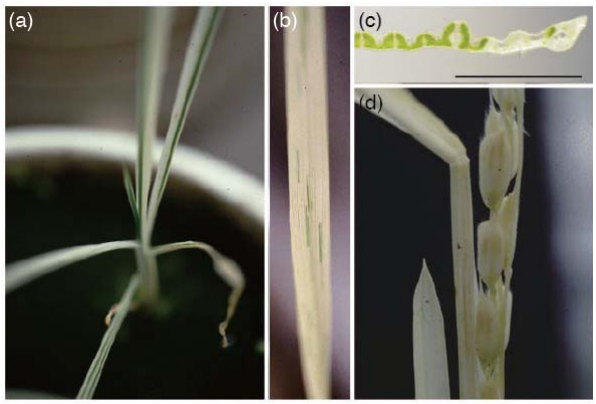

Figure 1. Phenotypes of swl1-v plants. Mutable albino plants with large green sectors (a) and with fine sectors (b). (c) Transverse section of the leaf blade. Scale bar = 1.0 mm. (d) Panicles.

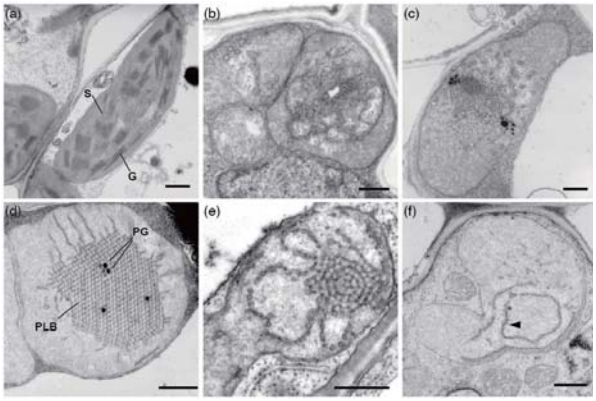

Figure 2. Plastid ultrastructures of WT and *swl1-stb* plants.
Chloroplasts from tertiary leaf blades of WT (a) and swl1-stb (b and c) seedlings grown under light. Etioplasts from the tertiary leaf blades of WT (d) and swl1-stb (e and f) seedlings germinated in the dark. G, grana thylakoid; S, stroma thylakoid; PG, plastoglobule; PLB, prolamellar body. The arrowhead indicates a ring-shaped structure formed from vesicles. Scale bars = 500 nm.

Publication List

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

● Hayashi-Tsugane, M., Takahara, H., Ahmed, N., Himi, E., Takagi, K., Iida, S., Tsugane, K., and Maekawa, M. A mutable albino allele in rice reveals that formation of thylakoid membranes requires SNOW-WHITE LEAF1 gene. Plant Cell Physiol. 2013 Oct. 21.

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 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. Studies in the past decade have demonstrated that 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. They are 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, functions 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 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 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.

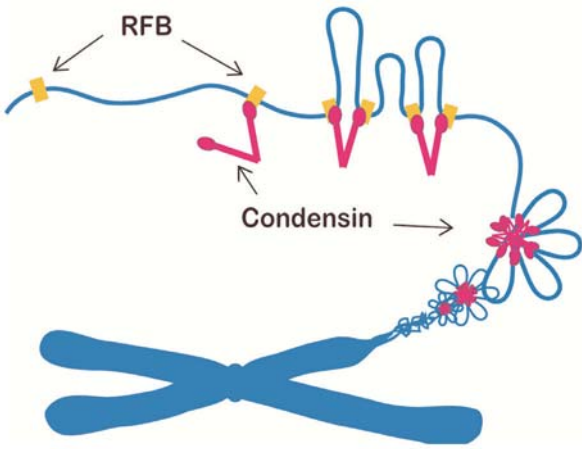

Figure 1. A schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This lead to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

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 developed software applications that are capable of describing cell dynamics out of 4D time-lapse imaging data sets by employing several approaches.

I. Automated cell tracking system

Epithelial morphogenesis in the fruit fly *Drosophila melanogaster* embryo is considered to be an excellent model for collective cell migrations. Drastic cell rearrangements lead drastic structural changes to build elaborate tubular organs such as the tracheal network. We developed 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). Obtained cell dynamics are subjected to comparative analyses among wild type and several mutants to unveil genetic programs underlying organogenesis.

II. Particle 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 track local features identified in individual images along a time-series to measure cell dynamics in organogenesis out of hazy images obtained by experimental constraints. This implementation detects local characteristics, such as uneven fluorescence, over the specimen and tracks these patterns along a time-series. By applying this tracking algorithm, we can successfully measure tissue deformation without recognizing individual cells such as in *Xenopus* neuroectoderm (Figure 2).

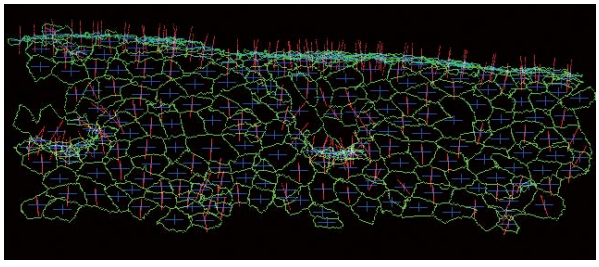

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

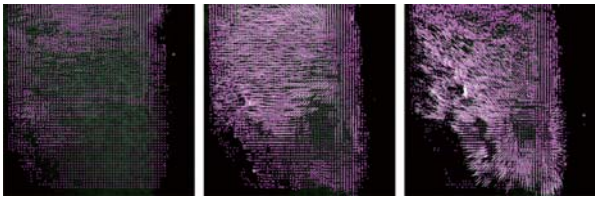

Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signal over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed microscopy.

III. A GUI application 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.jp/).

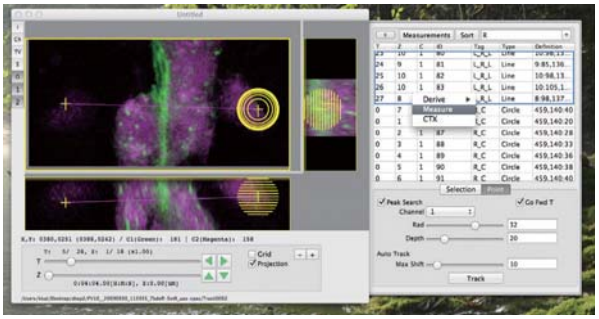

Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

LABORATORY OF BIOLOGICAL DIVERSITY

KIMORI Group

Specially Appointed Assistant Professor:
KIMORI, Yoshitaka

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.

I. Novel image processing method based on mathematical morphology

Image processing is a crucial step in the quantification of biomedical structures from images. As such, it is fundamental to a wide range of biomedical imaging fields. Image processing derives structural features, which are then numerically quantified by image analysis. Contrast enhancement plays an important role in image processing; it enhances structural features that are barely detectable to the human eye and allows automatic extraction of those features. To effectively recognize a region of interest, specific target structures must be enhanced while surrounding objects remain unmodified. A contrast enhancement technique which used mathematical morphology enables selective enhancement of target structures. Based on set theory, mathematical morphology applies shape information to image processing.

Mathematical morphology operates by a series of morphological operations, which use small images called structuring elements (typically, a single structuring element is used). The structuring element acts as a moving probe that samples each pixel of the image. Since the structuring element moves in a fixed direction across the image, some intricate images (in particular, those whose structural details contain a variety of directional characters) may not be properly processed. Consequently, an artifact in the shape of structuring elements may be generated at the object periphery. Since objects in biomedical images consist of delicate structural features, this drawback is an especially serious problem.

To overcome this problem, we have proposed an extension of conventional mathematical morphology called rotational morphological processing (RMP). The RMP based morphological filters have been applied to a wide variety of biomedical images, including electron micrographs, light micrographs and medical images such as mammographic images and chest X-ray images.

In this study, we have developed a novel RMP-based contrast enhancement method. The method uses a top-hat contrast operator, a well-known and commonly used morphological operation for extracting local features from a low-contrast image. Two types of top-hat operations exist; white top-hat (*WTH*) and black top-hat (*BTH*). *WTH* and *BTH* extract structures brighter and darker than the surrounding areas, respectively. In the proposed method, these RMP-based top-hat operators are computed in parallel. We applied the proposed method to enhancement of structural features in medical images: a mammographic image and a chest radiographic image. The performance of the method was subjectively and quantitatively evaluated by the contrast improvement ratio (*CIR*). The efficiency of the method was clearly demonstrated. Figure 1 shows chest radiographic images enhanced by the proposed method.

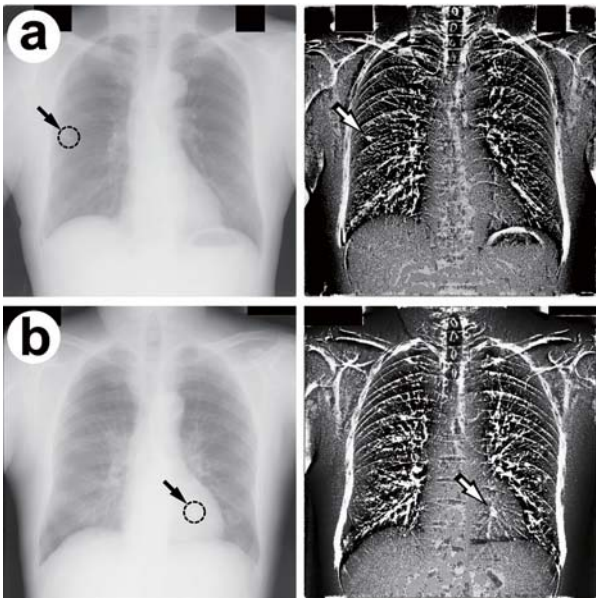

Figure 1. Enhancement of chest radiographic images. Left panel: Original chest radiographic images ((a): JPCLN80, (b): JPCLN152). Arrow in each image indicates nodule. Right panel: contrast enhanced images obtained by the proposed method. Chest radiographic images were obtained from the standard digital image database (Japanese Society of Radiological Technology).

Publication List

〔Original papers〕

- Kimori, Y. (2013). Morphological image processing for quantitative shape analysis of biomedical structures: effective contrast enhancement. J. Synchrotron Rad. *20,* 848-853.
- Kimori, Y., Baba, N., and Katayama, E. (2013). Novel configuration of a myosin II transient intermediate analogue revealed by quick-freeze deep-etch replica electron microscopy. Biochemical J. *450,* 23-35.