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. Eukaryotic cells employ Tor (target of rapamycin) protein kinase to recognize cellular nutrient conditions. 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).

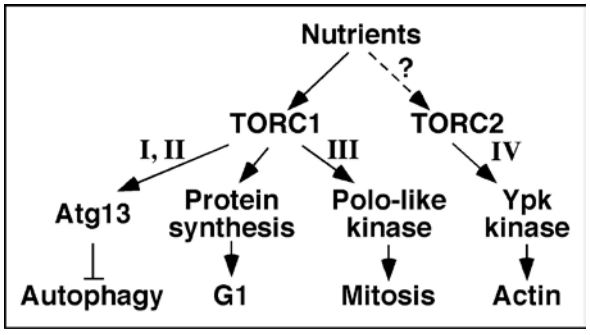

Figure 1. TOR signaling pathway of the budding yeast. Our group have found three branches of the TOR pathway.

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, such 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 is turned out to be a substrate of TORC1, in vivo activity of TORC1 can be monitored by phosphorylation state of Atg13. Various conditions and mutants have been examined to determine what kind of nutrients TORC1 recognizes and how nutrient signal is transmitted to TORC1.

III. Localization of Polo-like kinase is controlled by TORC1 to regulate mitotic entry.

It is well known that 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. We found that this mutant, as well as yeast cells treated with rapamycin, exhibit mitotic delay with prolonged G2. We further demonstrated that this G2-arrest phenotype is due to mislocalization and resultant inactivation of Cdc5, the yeast polo-kinase. These results suggest that TORC1 mediates G2/M transition via regulating polo-kinase.

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〕

● Yoshida, S., Imoto, J., Minato, T., Oouchi, R., Kamada, Y., Tomita, M., Soga, T., and Yoshimoto, H. (2011). A novel mechanism regulates H_2S and SO_2 production in *Saccharomyces cerevisiae*. Yeast *28*, 109-121.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor:	*OHNO, Kaoru*
Technical Assistants:	*ITO, Masako*
	NAKAMURA, Ryoko
	MATSUDA, Azusa
	FUJITA, Miyako

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).

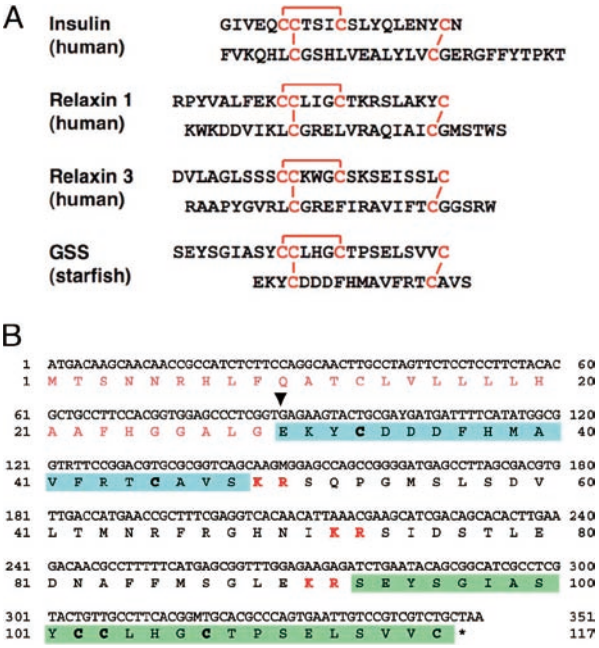

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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

Morning glories belong to the genus *Ipomoea* that is the largest group in the family *Convolvulaceae*. Of these, *I. 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.

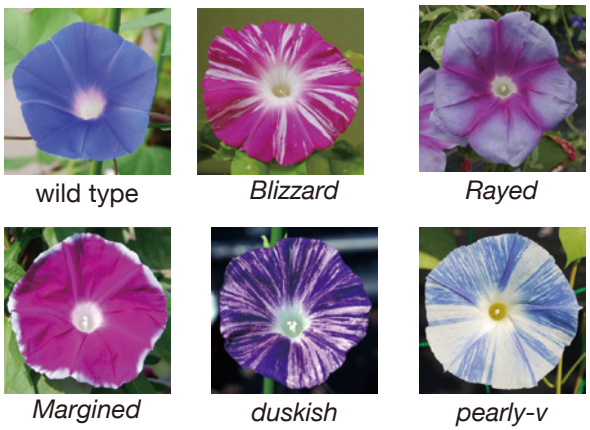

Figure 1. Flower phenotypes of the morning glories.

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 200 lines and 117,000 DNA clones.

Publication List

〔Original papers〕

- Higuchi, Y., Sage-Ono, K., Sasaki, R., Ohtsuki, N., Hoshino, A., Iida, S., Kamada H., and Ono, M. (2011). Constitutive expression of the *GIGANTEA* ortholog affects circadian rhythms and suppresses one-shot induction of flowering in *Pharbitis nil*, a typical short-day plant. Plant Cell Physiol. *52*, 638-650.
- Ohno, S., Hosokawa, M., Hoshino, A., Kitamura, Y., Morita, Y., Park, K.I., Nakashima, A., Deguchi, A., Tatsuzawa, F., Doi, M., Iida, S., and Yazawa, S. (2011). A bHLH transcription factor, *DvIVS*, is involved in regulation of anthocyanin synthesis in dahlia (*Dahlia variabilis*). J. Exp. Bot. *62*, 5105-5116.
- Ohno, S., Hosokawa, M., Kojima, M., Kitamura, Y., Hoshino, A., Tatsuzawa, F., Doi M., and Yazawa, S. (2011). Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. Planta *234*, 945-958.
- Saito, N., Tatsuzawa, F., Hoshino, A., Abe, Y., Ichimura, M., Yokoi, M., Toki, K., Morita, Y., Iida, S., and Honda, T. (2011). The anthocyanin pigmentation controlled by the *speckled* and *c-1* mutations of the Japanese morning glory. J. Japan. Soc. Hort. Sci. *80*, 452-460.

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

- Park, K.I., and Hoshino, A. A WD40-repeat protein controls proanthocyanidin and phytomelanin pigmentation in the seed coats of the Japanese morning glory. 2011 Dec. 28.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

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

An active nonautonomous DNA transposon, *nDart1-0*, belonging to the *hAT* superfamily, was identified. 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. The *nDart1* transposons tend to insert into the promoter, 5' UTR region, or exon of a gene, which suggests that the *nDart1*/*aDart1-27* system is a powerful tool for rice functional genomics. Furthermore, we are developing several *indica* lines bearing the active *nDart1*/*aDart1-27* system. These lines would effectively contribute to gene functional analysis and breeding for the *indica* rice varieties.

A rice mutant displaying a heterochronically elongated internode carries a 100 kb deletion

We have isolated a recessive rice mutant, designated as *indeterminate growth (ing)*, which displays creeping and apparent heterochronic phenotypes in the vegetative period with lanky and winding culms (Figure 1). Rough mapping and subsequent molecular characterization revealed that the *ing* mutant carries a large deletion, which corresponds to a 103 kb region in the Nipponbare genome, containing nine annotated genes on chromosome 3.

Figure 1. Phenotypes of the wild type and *ing* plants. A: Three-month-old wild-type plant (left) and ing mutant (right). The bars represent 50 cm.

Of these annotated genes, the *SLR1* gene encoding a DELLA protein is the only one that is well characterized in its function, and its null mutation, which is caused by a single base deletion in the middle of the intronless *SLR1* gene, confers a slender phenotype that bears close resemblance to the *ing* mutant phenotype. The primary cause of the *ing* mutant phenotype is the deletion of the *SLR1* gene, and the *ing* mutant appears to be the first characterized mutant having the entire *SLR1* sequence deleted. Our results also suggest that the deleted region of 103 kb does not contain an indispensable gene, whose dysfunction must result in a lethal phenotype (Figure 2).

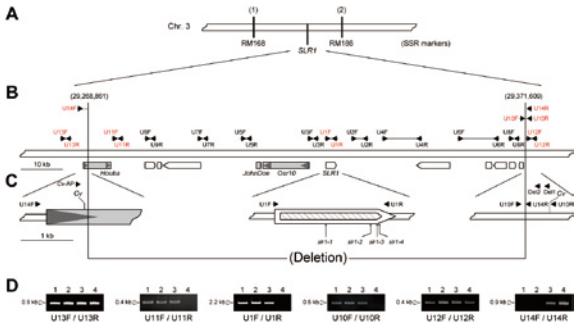

Figure 2. Characterization of the indeterminate growth (*ing*) allele. A: Rough mapping of the ing allele. B: Structure of the *ing* deletion. The large bracket indicates the deletion. C: Enlarged structures of the 5' region of *Houba*, SLR1, and the region of the 3' deletion junction. D: Detection of PCR-amplified bands with the indicated primers. Lane 1, Nipponbare; lane 2, *ING*/*ING*; lane 3, *ING*/*ing*; lane 4, *ing*/*ing*.

Examination of transpositional activity of *nDart1* at different stages of rice development

As a useful tool to elucidate gene functions, a rice transposon tagging line has been developed using an active endogenous DNA transposon, *nDart1*. It was highly desirable to evaluate the transposition timing and frequency of the *nDart1* elements during rice development to facilitate the generation of an efficient mutant isolation system. Comparison of the detected new insertions at different stages of rice development by transposon display analysis demonstrated that the last heading tiller carry a higher number of *nDart1* elements than the main culm. Moreover, it was revealed that the last heading tiller could produce progeny that carried more new insertions of *nDart1* elements, mainly as a result of the accumulation of somatic insertions in the parental plant. This report demonstrates that late tillers increase the probability of producing independent mutant lines.

Publication List

〔Original papers〕

- Hayashi-Tsugane, M., Maekawa, M., Kobayashi H., Iida, S., and Tsugane, K. (2011). A rice mutant displaying a heterochronically elongated internode carries a 100 kb deletion. J. Genet. Genomics *38*, 123-128.
- Hayashi-Tsugane, M., Maekawa, M., Qian, Q., Kobayashi H., Iida, S., and Tsugane, K. (2011). Examination of transpositional activity of *nDart1* at different stages of rice development. Genes Genet. Syst. *86*, 215-219.

〔Review article〕

- Maekawa, M., Tsugane, K., and Iida, S. (2011). Effective contribution of the *nDart* transposon-tagging system to rice functional genomics. Adv. Genet. Res. *4*, 259-272.

LABORATORY OF BIOLOGICAL DIVERSITY

YAMAGUCHI Group †

Assistant Professor: *YAMAGUCHI, Takahiro*
Postdoctoral Fellow: *NUKAZAWA, Akira*
Technical Assistant: *YAMAGUCHI, Chinami*

Angiosperm leaves generally develop as bifacial structures with distinct adaxial and abaxial identities. However, several monocot species, such as iris and leek, develop "unifacial leaves", in which leaf blades have only an abaxial identity (Figure 1). We are focusing on unifacial leaf development and evolution to understand genetic mechanisms behind diversity and evolution of organismal morphology.

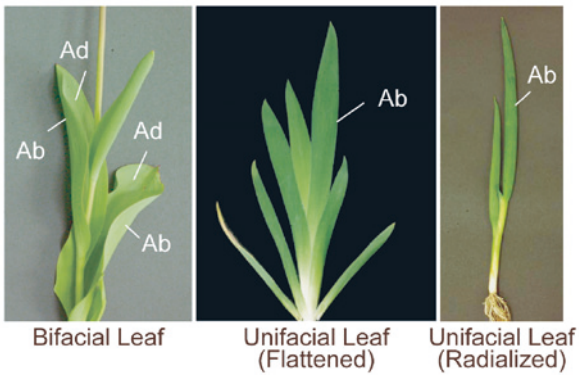

Figure 1. Bifacial and unifacial leaf structures. Ad, Adaxial side; Ab. Abaxial side.

I. Abaxialization of unifacial leaves

The development and evolution of unifacial leaves have long been matters of debate. However, nothing has been studied at the molecular genetic level. We focused on the genus *Juncus* as a model to study the evolution and development of unifacial leaves. *Juncus* contains species with a wide variety of leaf forms and is amenable to molecular genetic studies. We first characterized unifacial leaf development by investigating gene expression patterns of adaxial and abaxial determinants. As a result, we demonstrated that the unifacial leaf blade is abaxialized at the gene expression level and revealed that dominant abaxial activity leads to the unifacial leaf development.

II. Flattening of unifacial leaves

In bifacial leaves, adaxial–abaxial polarity is required for leaf blade flattening, whereas many unifacial leaves become flattened although their leaf blades are abaxialized (Figure1). This indicates independent mechanisms underlying flattened leaf blade formation in bifacial and unifacial leaves.

Using two closely related *Juncus* species, *J. prismatocarpus*, with flattened unifacial leaves, and *J. wallichianus*, with radialized unifacial leaves, we revealed that *DL* expression levels and patterns correlate with the degree of laminar outgrowth. Genetic and expression studies using interspecific hybrids of the two species revealed that the *DL* locus from *J. prismatocarpus* flattens the unifacial leaf blade and expresses higher amounts of *DL* transcripts. Thus, *DL* is a key gene in the flattening of the unifacial leaf blade. Interestingly, *DL* plays a distinct role in promoting midrib formation during bifacial leaf development. We suggest that morphological convergence of flattened leaf blades in unifacial leaves has occurred via the recruitment of *DL* function, which plays a similar cellular but distinct phenotypic role in monocot bifacial leaves (Figure 2).

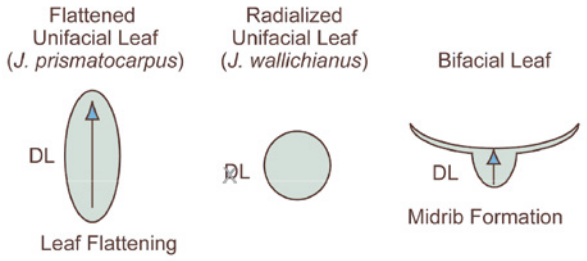

Figure 2. Mechanism of leaf blade flattening in unifacial leaves.

III. Leaf central–marginal and distal–proximal polarity specification

The mechanisms that regulate the central–marginal leaf polarity remain largely unknown. We discovered that the central–marginal polarity differentiates in the flattened leaf blade of *J. prismatocarpus*, but not in the radialized leaf blade of *J. wallichianus*. This indicates that leaf blade flattening is a key that triggers central–marginal leaf polarity differentiation. We also found the possibility that the plant hormone auxin is asymmetrically distributed in the flattened leaf primordia and induces expressions of central-marginal polarity determinants.

We also revealed that the *ROTUNDIFOLIA4* (*ROT4*) gene that encodes a plant-specific small peptide controls cell proliferation along distal-proximal axis during leaf development in *Arabidopsis thaliana*. We identified the 32 residues-long core functional region of ROT4 and showed that ROT4 acts cell autonomously without proteolytic processing (Ikeuchi et al., 2011).

Publication List

〔Original paper〕

● Ikeuchi, M., Yamaguchi, T., Kazama, T., Ito, T., Horiguchi, G., and Tsukaya, H. (2011). ROTUNDIFOLIA4 regulates cell proliferation along the body axis in Arabidopsis shoot. Plant Cell Physiol. *52*, 59–69.

†: This group was closed on 30 September, 2011.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: *JOHZUKA, Katsuki*
Technical Assistants: *ISHINE, Naomi*
MATSUZAKI, Yoko

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 created during DNA replication. Any abnormality in this process lead to segregation error or aneuploidy, resulting in cell lethality. Studies in the past decade have demonstrated that chromosome condensation is mainly achieved by condensin, a multi-subunit protein complex widely conserved from yeast to human.

Our major 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 genome during mitosis. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. We further discovered the multiple proteins interaction network recruits condensin to the RFB site.

I. Maintenance of the rDNA stability by condensin

Due to its tandem repetitive structure, the rDNA locus is unstable because of frequent homologous recombination events within the rDNA repeat. Nevertheless, most cells can maintain rDNA stability, as indicated by their relatively constant copy numbers of the repeats. A key recombination protein, Rad52 is normally localized in the nucleus but is excluded from the nucleolus. This exclusion of a recombination protein may contribute to maintaining rDNA stability. In condensin mutants, however, aberrant segregation of the rDNA locus is frequently observed and its copy numbers are dramatically decreased. We found that the Rad52 protein was excluded from the nucleolus during the mitosis in *YCS4* cells, whereas it invaded into the nucleolus in *ycs4-1* mutant cells (Figure 1). This result indicates that condensin protects from invasion of recombination enzyme during mitosis. It is suggested that rDNA condensation supports the exclusion of recombination machinery, resulting in avoidance of the accumulation of recombination intermediates within the rDNA locus, thus contributing to maintaining rDNA stability and faithful segregation.

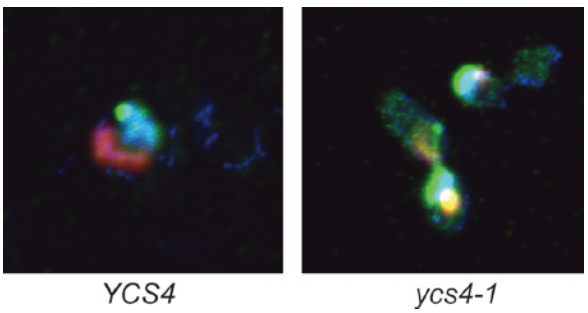

Figure 1. Nucleolar localization of Rad52 protein during mitotic phase. The wild-type (*YCS4*) and condensin mutant (*ycs4-1*) cells were arrested at metaphase by interfering with the polymerization of microtubules (nocodazole). Nucleolar organizing protein (Nop56) was detected as red and Rad52 protein was detected as green colors, respectively. In the *ycs4-1* mutant, Rad52 invades in the nucleolus (yellow).

II. Condensin-dependent chromatin folding

The RFB site, which consists of a ~150bp DNA sequence, acts as the strongest cis-element for condensin recruitment onto chromatin. If the RFB sequence 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 at an interval of 15kb distance in the cell with complete deletion of chromosomal rDNA repeat. Using such strain, condensin-dependent chromatin interaction between two RFBs was examined by chromosome conformation capture (3C) assay. We found condensin-dependent interaction between 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 2). It is thought that condensin-dependent chromatin folding is one of the basic molecular processes of chromosome condensation.

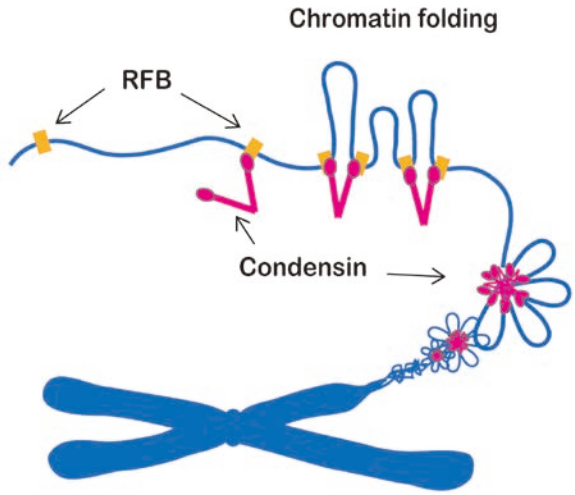

Figure 2. A Schematic model of chromosome condensation. Condensin causes chromatin interactions between adjacent binding sites (RFB, for example). This lead to a folding of chromatin fiber between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

WATANABE Group †

Assistant Professor: *WATANABE, Takaaki*
Technical Assistant: *YONEZAWA, Harumi*

Genomes have been dynamically evolving and are continually changing during development, and through diseases and environmental stress. One type of genome alteration, gene amplification, is involved in various biological phenomena, such as malignant progression of cancer, resistance to insecticides and anticancer drugs, and gene evolution. We are addressing the molecular mechanisms underlying gene amplification from a variety of perspectives.

I. Model systems for studying mechanisms for gene amplification

Long series of studies have shown that DNA double-strand breaks and inverted repeats play an important role in gene amplification. However, details of the molecular mechanisms remain to be determined. This is because previous approaches to understanding the mechanisms were based on the structural analysis of complex end products and very few model systems are available that allow chromosomal engineering and genetic analysis.

To better understand the molecular mechanisms, we have developed a new approach in which we design amplification processes and test whether the processes can produce the amplification seen in nature. Previously, we constructed a system designed to induce a rapid amplification mode, double rolling-circle replication, (DRCR, Figure 1A) via chromosomal breaks induced by site-specific endonuclease (EMBO J, 2005). This system produced intra-/extra-chromosomal products resembling those seen in mammalian cells; homogeneously staining regions (HSR) and double minutes (DMs). This result strongly suggested that amplification in mammalian cells involves DRCR.

We next examined whether recombinational processes coupled with replication can induce gene amplification via DRCR, using a distinct process, Cre-lox site-specific recombination. Here, we inferred that, if Cre recombination coupled with replication occurs, the replication fork makes an additional copy of the replicated region (Figure 1B); and that the processes from two pairs of lox sites could induce DRCR (Figure 1C). In this study, we successfully detected HSR/DM-type amplification products in yeast and Chinese hamster ovary (CHO) cells (Figure 1D and 1E). Surprisingly, over 10% of the Cre recombination-induced yeast cells undergo gene amplification. In addition, scattered-type products were also found (Figure 1F), which are frequently seen in cancer cells. From these results, we reasoned that DRCR and convergent replication are centrally involved in the amplification of drug-resistance genes and oncogene. This system can serve as a good model for amplification in mammalian cells and contribute to a better understanding of oncogene amplification and development of anticancer strategies in future.

II. Intensive rearrangement in amplified region

In amplified chromosomal regions intensive chromosome rearrangements are frequently observed, leading to an increase in the gene copy number and to a decrease in size of the amplification unit. In oncogene amplification, the complex patterns of amplification generated by the rearrangements are closely associated with poor prognosis in cancer. Interestingly, we have observed the rearrangement in all our DRCR systems.

To explore the link between the rearrangements and the DRCR process, we constructed a system that can turn on or off the occurrence of DRCR, using yeast 2μ plasmid. This system demonstrated that inversions, deletions, or duplications could be intensively induced in a DRCR-dependent manner. This result suggests that DRCR may cause the rearrangements in amplification in nature. We proposed a model in which DRCR markedly stimulates recombinational events.

III. A structural platform for gene amplification

Based on our results, we now focus on a type of genomic structure consisting of two sets of inverted repeats, designated double IR. This structure is found in the human genome and can be observed in the early stages of gene amplification. In yeast, the double IR could induce gene amplification. Now we try to construct the double IR in CHO cells and to perform its detailed functional analysis in yeast.

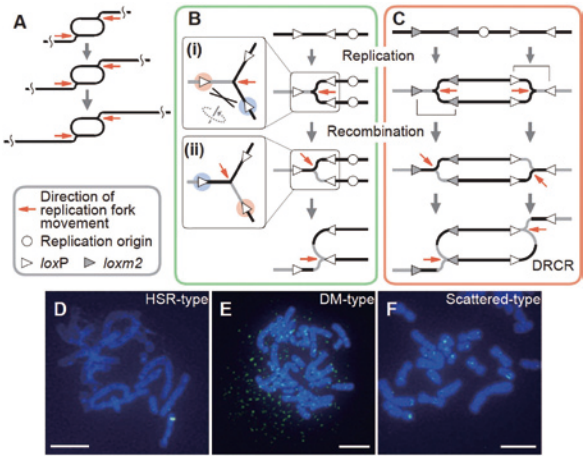

Figure 1. DRCR process, recombinational process coupled with replication, and amplification products in CHO cells.

Publication List

〔Original papers〕

● Okamoto, H., Watanabe, T., and Horiuchi, T. (2011). Double rolling circle replication (DRCR) is recombinogenic. Genes Cell *16*, 503-513.
● Watanabe, T., Tanabe, H., and Horiuchi, T. (2011). Gene amplification system based on double rolling-circle replication as a model for oncogene-type amplification. Nucleic Acids Res. *39*, e106.

†: This group was closed on 31 March, 2012.