DIVISION OF GENOME DYNAMICS

Professor:	*HORIUCHI, Takashi*
Associate Professor:	*KOBAYASHI, Takehiko*
Research Associate:	*JOHZUKA, Katsuki*
Technical Staff:	*MOROOKA, Naoki*
JSPS Research Fellow:	*CUI, Tailing*
Postdoctoral Fellows:	*KODAMA, Ken-ichi*
	AUSTEN, RD Ganley
	OHSUMI, Katsufumi
	SERIZAWA, Naomi
	WATANABE, Takaaki
Visiting Scientist:	*UJIIE, Yoshifumi*
Graduate Student:	*ITAZU, Masako*
Technical Assistant:	*IGAWA, Emi*
Secretary	*MIKAMI, Yuriko*

The genomes of higher organisms contain significant amounts of repetitive sequences which, in general, are unstable. At present, neither the physiological function(s) of repeated sequences nor the mechanism controlling instability is fully understood. To clarify these aspects, we are pursuing several themes using *Saccharomyces cerevisiae* and in 2005 the following four subjects have advanced our knowledge of the dynamics of the genome: (1) identification of non-coding functional elements possibly involved in amplification and stability of the rRNA repeated genes in yeast, (2) a finding of recombination regulation by transcription-induced cohesin dissociation in rDNA, (3) a finding of a new role of condensin in maintaining a long repeated structure of rDNA, (4) development of *in vivo* artificial gene amplification systems.

I. Identifying gene-independent non-coding functional elements in the yeast ribosomal DNA by phylogenetic footprinting

Sequences involved in the regulation of genetic and genomic processes are primarily located in non-coding regions. Identifying such cis-acting sequences from sequence data is difficult because their patterns are not readily apparent, and, to date, identification has concentrated on regions associated with gene-coding functions. We used phylogenetic footprinting to look for gene-independent non-coding elements in the ribosomal RNA gene (rDNA) repeats from several *Saccharomyces* species. Similarity plots of ribosomal intergenic spacer alignments from six closely related *Saccharomyces* species allowed the identification of previously characterized functional elements, such as the origin-of-replication (ARS) and replication-fork barrier (RFB) sites, demonstrating that this method is a powerful predictor of non-coding functional elements. Seventeen previously uncharacterized elements, showing high level of conservation, were also discovered. The conservation of these elements suggests that they are functional, and we demonstrate the functionality of two classes of these elements, a putative bidirectional non-coding promoter (later we named E-pro) and a series of conserved peaks with matches to the origin-of-replication core consensus. Our results paint a comprehensive picture of the functionality of the *Saccharomyces* ribosomal intergenic region and demonstrate that functional elements not involved in gene-coding function can be identified by using comparative genomics based on sequence conservation.

II. Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats in yeast

In most eukaryotic organisms, the rDNAs are clustered in long tandem repeats on one or a few chromosomes. Although the total number of these chromosomal rDNA repeats appears to be maintained at a level appropriate for each organism, genes with such a repeated structure are in general thought to be unstable because of a high frequency of recombinational events. Thus, it might be expected that organisms have developed systems to regulate recombination within rDNA repeats.

In the yeast *S. cerevisiae*, approximately 150 copies of rDNA are maintained on chromosome XII. Recombinational events within the rDNA repeats in normal growing yeast cells appear to be mostly mediated by a *FOB1*-dependent system. *FOB1* is the gene required for fork blocking activity at RFB site, recombination in the rDNA region, and expansion/contraction of rDNA repeats. The latter two activities are likely to be triggered by double-strand breaks at the RFB site and repair of the breaks via gene conversion. On the other hand, the *SIR2* gene plays an important role in decreasing the frequency of recombination in yeast rDNA. Sir2p is a protein required for transcriptional silencing at three yeast chromosomal regions, silent mating type loci, telomeres, and rDNA. It is generally believed that Sir2p, perhaps through its NAD^+-dependent histone deacetylase activity, plays an essential role in forming a higher order of repressive chromatin structure – heterochromatin - which prevents general access of the PolII machinery and some other macromolecules, thus causing silencing as well as decreasing recombination in the chromosomal rDNA repeats. Therefore, mutations in gene *SIR2* increase recombination within rDNA repeats as assayed by marker loss or extrachromosomal rDNA circle formation.

We examined the mechanism involved in the increased frequency of recombination in rDNA repeats that is observed in mutants defective in *SIR2* functions. We measured the frequency of *FOB1*-dependent arrest of replication forks, consequent DNA double-strand breaks, and formation of DNA molecules with Holliday junction structures, and found no significant difference between *sir2Δ* and *SIR2* strains. Formal genetic experiments measuring mitotic recombination rates within individual rRNA genes also showed no significant difference between these two strains. Instead, we found a significant decrease in the association of the cohesin subunit Mcd1p (Scc1p) to the rDNA in *sir2Δ* relative to *SIR2* strains. From these and other experiments, we conclude that *SIR2* prevents unequal sister-chromatid recombination, probably by forming special cohesin structures, without significant effects on recombinational events within

individual rRNA genes.

We found that bidirectional transcription actually occurs from an assuming non-coding bidirectional promoter (E-pro) within the rDNA and is absolutely required for amplification of rDNA. E-pro transcription stimulates the dissociation of cohesin, a DNA binding protein complex that suppresses sister-chromatid-based changes in rDNA copy number. This transcription is regulated by the silencing gene, *SIR2*, and by copy number. These results suggested a model of amplification regulation where transcription of E-pro stimulates unequal recombination by disrupting cohesin association in the rDNA, thus allowing for a change in copy number (Figure 1). Transcription-induced cohesin dissociation may be a general mechanism of recombination regulation.

III. Condensin loaded onto the rDNA in a *FOB1*-dependent fashion to avoid contraction of a long repeat

An average of 200 copies of the ribosomal RNA genes (rDNA) are clustered in a long tandem array in *S. cerevisiae*. *FOB1* is known to be required for expansion/contraction of the repeats by stimulating recombination, thereby contributing in the maintenance of average copy number. In Δ*fob1* cells, the repeats are still maintained without any fluctuation of copy number, suggesting another unknown system acts to prevent repeats contraction. We attempted to understand the putative second *unknown* system by which the long rDNA array is maintained by isolating mutants carrying unstable, shortened rDNA tandem array in a *Δfob1* background. We

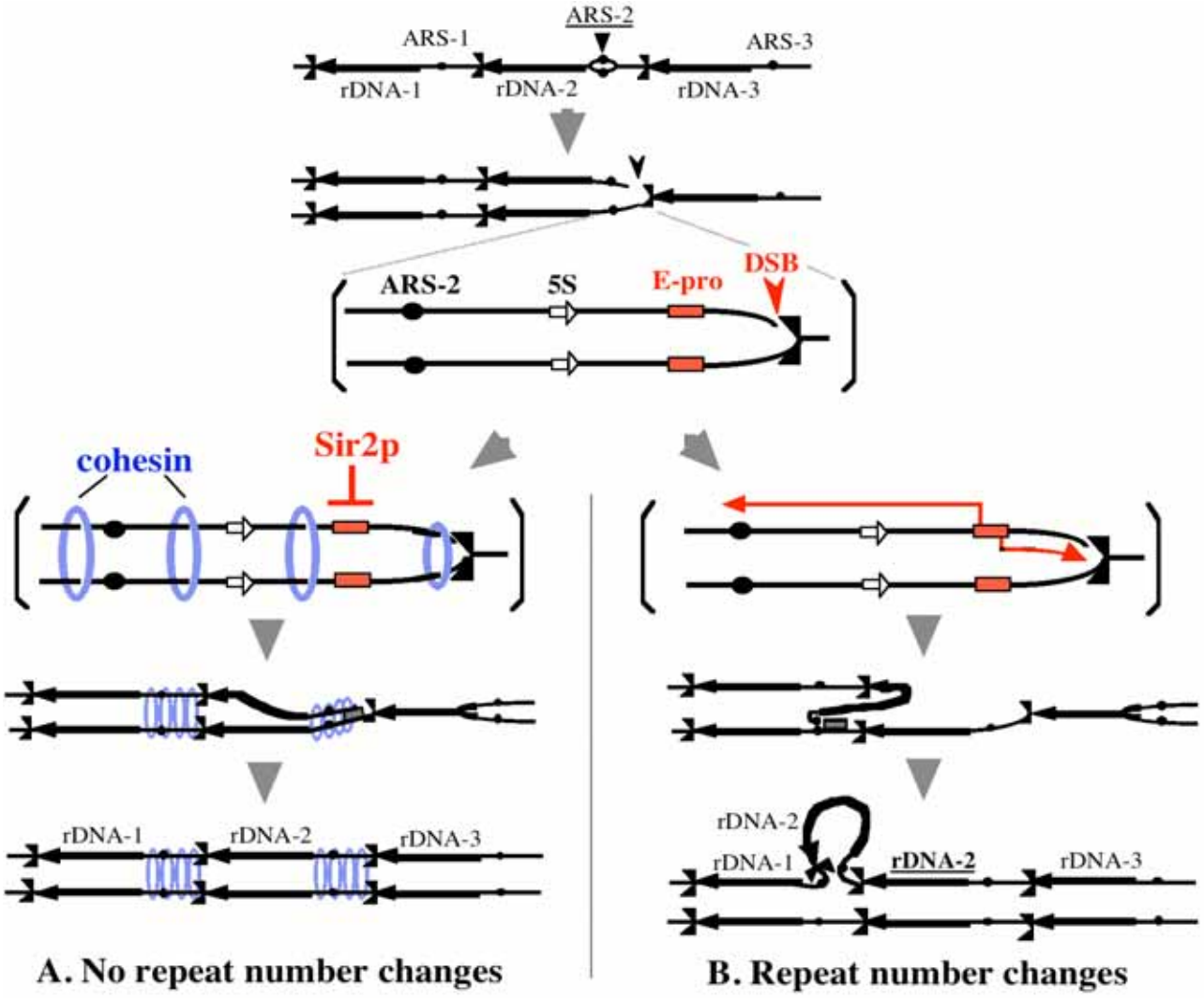

Figure 1. Transcription-induced cohesin dissociation model of rDNA amplification. (A) In normal situation, such as wild-type rDNA copy number, SIR2 repress E-pro activity, allowing cohesin to associates throughout the IGS (intergenic spacer; NTS1). DSBs (double stranded breaks), formed by replication forks pausing at the RFB site, are repaired by equal sister-chromatid recombination, with no change in the rDNA copy number. (B) When *SIR2* repression is removed, such as sir2 mutation or low copy number, E-pro becomes active and transcription displaces cohesin. Unequal sister chromatids can then be used as templates for DSB repair, resulting in changes in the rDNA copy number. Lines represent single chromatids (double stranded DNA). The IGS in which the replication fork is paused is expanded in the bracket.

found that condensin mutants showed severe contraction of rDNA tandem array in a *Δfob1* cells, but not in *FOB1*$^{+}$ cells, thereby suggesting that condensin acts to maintain long repeats with *FOB1* in a functionally complemented fashion. We also found that condensin association with the rDNA was localized to the replication fork barrier (RFB) site in a *FOB1*-dependent fashion. Surprisingly, condensin association with the RFB site was established during S phase and maintained until anaphase. These results indicate that *FOB1* acts in a novel role to prevent repeats contraction by regulating condensin association, and suggest a link between replication termination, and chromosome condensation and segregation.

IV. A novel gene amplification system in yeast based on double rolling-circle replication

Gene amplification is involved in various biological phenomena such as cancer development and drug resistance. However, the mechanism is largely unknown because of the complexity of the amplification process. We developed a gene amplification system in *S. cerevisiae* that is based on double rolling-circle replication (DRCR), utilizing break-induced replication (BIR). This system produced three types of amplification products. Type-1 products contain 5–7 inverted copies of the amplification marker, *leu2d*. Type-2 products contain 13 to ~100 copies of *leu2d* (up to ~730 kb increase) with novel arrangement present as randomly oriented sequences flanked by inverted *leu2d* copies. Type-3 products are acentric multi-copy mini-chromosomes carrying *leu2d*. Structures of type-2 and -3 products resemble those of homogeneously staining region (HSR) and double minutes (DMs) of higher eukaryotes, respectively. Interestingly, products analogous to these were generated at low frequency without deliberate DNA cleavage. These features strongly suggest that the processes described here may contribute to natural gene amplification in higher eukaryotes.

Publication List:

Original papers

Ganley, A.R., Hayashi, K., Horiuchi, T., and Kobayashi, T. (2005). Identifying gene-independent noncoding functional elements in the yeast ribosomal DNA by phylogenetic footprinting. Proc. Natl. Acad. Sci. USA *102*, 11787-11792.

Kobayashi, T., and Ganley, A.R. (2005). Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. Science *309*, 1581-1584.

Watanabe, T., and Horiuchi, T. (2005). A novel gene amplification system in yeast based on double rolling-circle replication. EMBO J. *24*, 190-198.