Genes Genet. Syst. (2006) **81**, p. 155–161

# Strategies to maintain the stability of the ribosomal RNA gene repeats

# –Collaboration of recombination, cohesion, and condensation–

Takehiko Kobayashi*

*National Institute for Basic Biology and The Graduate University for Advanced Studies SOKENDAI, School of Life Science, 38 Nishigonaka, Myodaijicho, Okazaki, 444-8585 Japan*

*(Received 17 May 2006, accepted 29 May 2006)*

Ribosomal RNA gene repeats (rDNA) are one of the most characteristic regions in eukaryotic chromosomes. The repeats consist of more than 100 tandem units occupying large part of the chromosome in most of organisms. Cells are known to deal with this "unusual domain" in a unique manner. In this review, I will summarize work on rDNA repeat maintenance, focusing mainly on work done by our group, and show that the maintenance mechanism operates by a collaboration of recombination, sister-chromatid cohesion, and chromatin condensation.

**Key words:** ribosomal RNA gene, recombination, cohesion, condensation, gene amplification, genome stability, noncoding transcription

## INTRODUCTION

Recombination is found throughout life, playing roles in DNA repair, chromosome segregation, and rescue of stalled replication forks. Despite these positive roles, unsuitable recombination is also known to cause genetic instability. In particular, repeated genes families such as the ribosomal RNA genes (rDNA) are one of the most fragile sites for deletional recombination. Even though the rDNA is susceptible to this kind of instability, each organism is known to maintain a specific repeat copy number of rDNA (Table 1), thereby indicating presence of a mechanism for the maintenance of copy number. This is reinforced by the fact that the copy number is tightly regulated, and if repeats are deleted or inserted, copy number quickly recovers to the wild-type level (Rodland and Russell, 1982; Kobayashi et al., 1998). Currently we know two ways by which copy number is maintained. One way is by gene amplification after deletional recombination in the repeats. This amplification is promoted by replication-coupled recombination. The other way is repression of copy number change by silencing, similar to how heterochromatin functions in higher eukaryotic cells, when the copy number is normal. This repression prevents unequal sister-chromatid recombination that changes the copy number. These amplification and repression events are the main mechanism to keep rDNA copy number. Additionally, condensation of rDNA is important for proper segregation of the rDNA chromosome in mitotic (M) phase. In this review I summarize our current understanding of how theses processes contribute to maintenance of copy number and repeats stability.

### rDNA is a "department store" of genomic elements

The rDNA encodes genes for structural RNA components of the ribosome, that serves as a factory for translation of mRNA. Because a cell requires so many rRNA molecules (making up ~70% of total RNA molecules), most organisms have multiple copies of the rDNA (Table 1). In the yeast *Saccharomyces cerevisiae*, there are ~150 tandemly repeated copies of the rDNA on chromosome XII (chr XII) in a haploid cell. The rDNA occupies ~60% of the chromosome, therefore the repeats are expected to contain most of the genomic elements necessary for proper chromosome function. An rDNA repeating unit (9.1 kb, Fig. 1) consists of two rRNA genes (35S and 5S) and two intergenic spacers (IGS1 and IGS2, formerly called NTS1 and NTS2.). The 35S and 5S rRNA genes are transcribed specifically by RNA polymerase I and III (Pol I and III), respectively. Interestingly, a mitochondrial protein, Tar1p, is encoded on the antisense strand of the 35S rDNA (Coelho et al., 2002). *TAR1* is

Edited by Hideo Shinagawa

* Corresponding author. E-mail: koba@nibb.ac.jp

Table 1. The copy number of the large rDNA in various organisms (haploid)

| | |
|---|---|
| *E. coli* | 7 (Ellwood and Nomura, 1982) |
| *Saccharomyces cerevisiae* | 150 (Kobayashi et al., 1998) |
| *Drosophila melanogaster* | 240 (Tartof, 1979) |
| *Xenopus laevis* | 600 (Wallance and Birnstiel, 1966) |
| *Homo sapiens* | 350 (Sakai et al., 1995) |
| *Arabidopsis thaliana* | 570 (Pruitt and Meyerowitz, 1986) |
| *Pisum sativum* (pea) | 3,900 (Ingle et al., 1975) |

For review see Long and Dawid (1980)

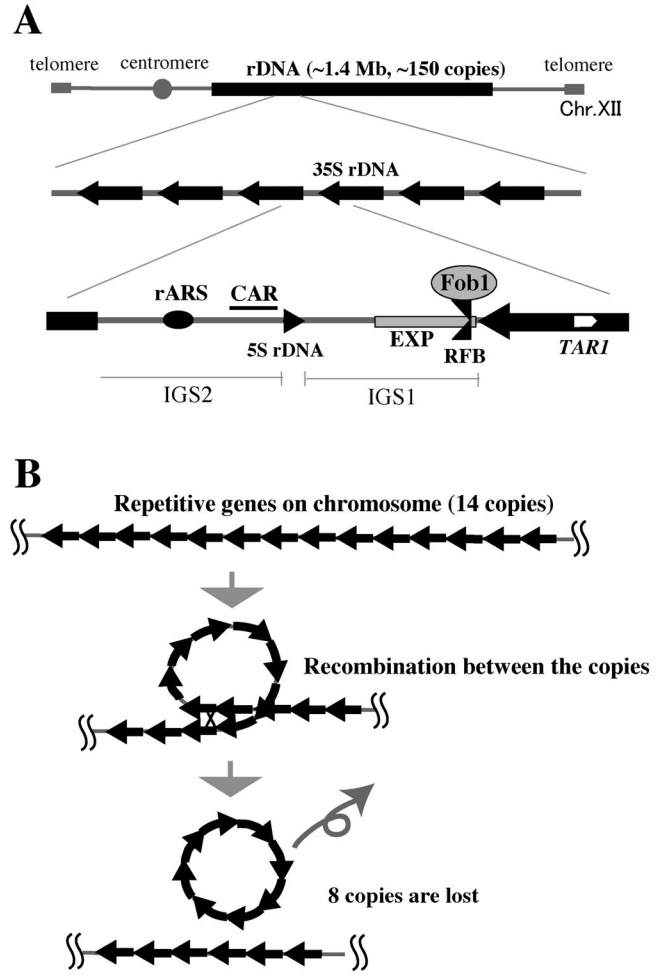


Fig. 1. (**A**) Structure of the rDNA of *Saccharomyces cerevisiae*. The rDNA occupies ~60% of chr XII in *S. cerevisiae*. The 35S and 5S rRNA genes, rDNA intergenic spacers (IGS1 and 2), origin of replication (*rARS*), replication fork barrier (RFB), EXP, *TAR1*, and cohesin associating region (CAR) are indicated. Arrows show the direction of transcription. (**B**) Recombination between the repeats reduces the copy number. In this case, the repeats lose eight copies.

transcribed by RNA polymerase II (Pol II). In the IGS2, there is a replication origin, *rARS* (for review see Brewer and Fangman, 1991), and a cohesin associating sequence, CAR (Laloraya et al., 2000). In the IGS1, there is a replication fork barrier site, RFB (Kobayashi et al., 1992, Brewer et al., 1992). Fob1p specifically associates with the RFB and inhibits rightward replication but not the other direction in Fig. 1A (Kobayashi, 2003). It is known that condensin and a silencing protein, Sir2p, associate with the RFB region (Freeman et al., 2000; Huang and Moazed, 2003: Johzuka et al., 2006). Thus, the rDNA repeats contain many elements found generally in the genome.

**Mechanism of gene amplification in the rDNA repeats**

***Analysis of trans-acting factors.*** Repeated genes like the rDNA are instable because of deletional recombination among the repeats (Fig. 1B). Therefore, gene amplification is expected to occur after copy number loss to return the copy number to its proper level. To understand the mechanism of amplification, we established an assay system, the "amplification assay" (Kobayashi et al., 1998). In this assay we used a RNA polymerase I (Pol I) mutant that carries approximately half (~80) of the wild type level of rDNA repeat units, and upon introduction of the missing Pol I gene (*RPA135*), the number of rDNA repeats returns back to the wild type level by amplification. Therefore, by combining the Pol I mutation with mutation in other genes of interest, we were able to determine what genes are necessary for the amplification that is induced by introduction of the Pol I gene. By this assay, we found that genes responsible for double strand break (DSB) repair, such as *RAD52* and *MRE11*, are required for the amplification (Kobayashi et al., 2004). In addition, the *FOB1* gene that encodes the replication fork blocking protein, Fob1p, is also required (Kobayashi et al., 1998). Using this information, we present a model detailing how these gene products contribute amplification, as shown in Fig. 2. In this model, the RFB/Fob1p complex is working as a recombinational hotspot that causes DNA double strand breaks (DSBs) that are the initiating event of recombination. In fact, such DSBs have been identified (Kobayashi et al., 2004: Burkhalter and Sogo, 2004: Weitao et al., 2003). These DSBs are then repaired by recombination. If the broken end recombines with an unequal sister-chromatid, some copies are replicated twice and the copy number increases (See Fig. 2-d). In contrast, if the broken end chooses unreplicated chromatin as the partner for recombination (*e.g.* around rARS-3 in Fig. 2-b), copies are skipped from replication (35S rDNA-③ in this case) and the copy number reduces. Therefore, this model explains both amplification and reduction of rDNA copy number through unequal sister chromatid recombination. As Pol I transcription is required for rDNA amplification, the transcription seems to bias the recombination towards increasing the copy number by selection of the replicated region (around rARS-1 in Fig. 2-c), as a recombination target, although

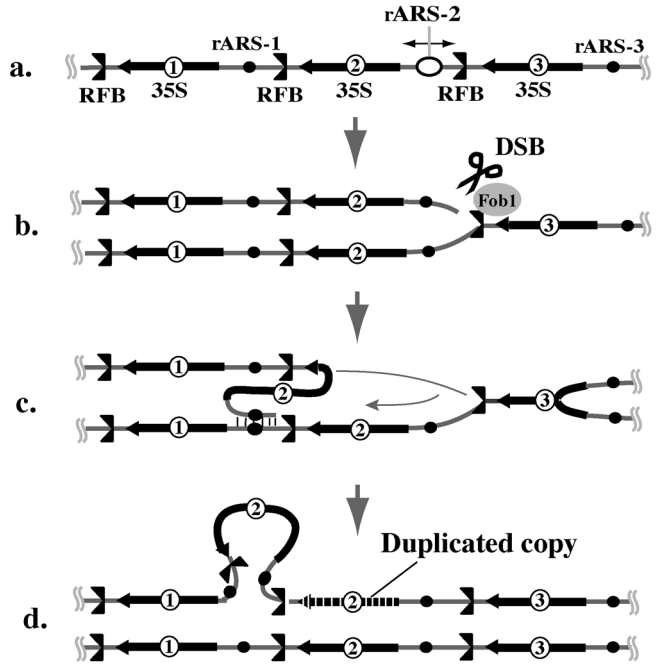


Fig. 2. The RFB/Fob1p dependent amplification model. Three copies out of 150 are shown. Individual lines represent chromatids with double-stranded DNA. In this model, DNA replication starts from one of the *rARS* (rARS-2) bidirectionally (a). A rightward replication fork is arrested at the RFB site and this arrest stimulates a DNA double strand break (DSB; indicated by the scissors in b). A strand invasion into a homologous sister duplex (a downstream sister chromatid near rARS-1 in this example) takes place (c) and a new replication fork is formed. The new replication fork meets with the leftward moving replication fork from the 35S rDNA-③ direction, resulting in formation of two sister chromatids, one of which gains an extra copy of rDNA, indicated as striped 35S rDNA-② (d). The model was originally proposed by Kobayashi et al. (1998).

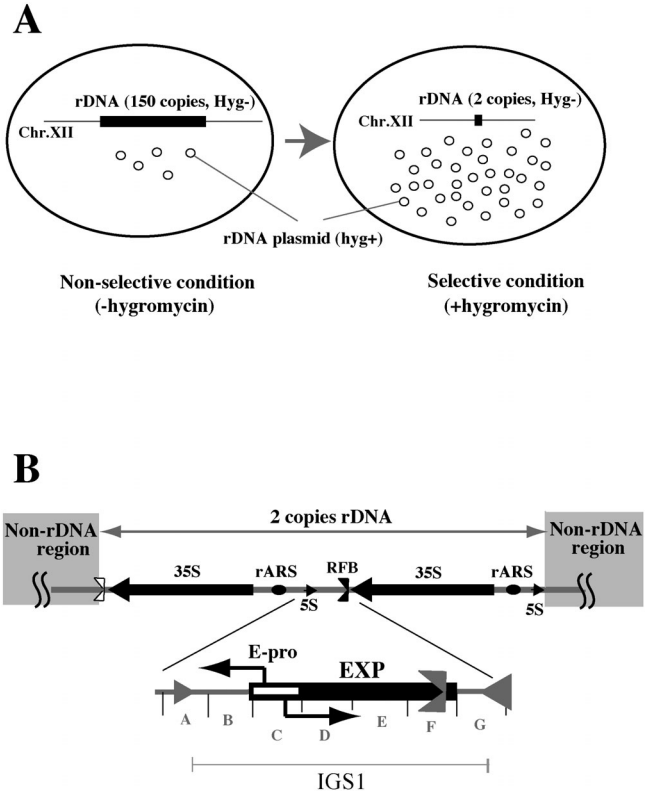


Fig. 3. (**A**) Construction of the two-rDNA copy strain. A plasmid (pRDN-hyg1) that carries a single rDNA repeat with a recessive hygromycin-resistant mutation in the 35S rDNA is introduced into a wild type strain that has 150 rDNA copies. The resultant transformant is subjected to selection on hygromycin. Because the wild type allele is dominant to the mutant *hyg1* allele, hygromycin-resistant mutants selected in this way are expected to have lost most of the chromosomal rDNA repeats by spontaneous deletional recombination. Among them, a strain that has only two rDNA copies is selected. (**B**) Structure of the two-rDNA copies strain and detection of *cis*-essential sequences for the amplification. The boundaries of the repeats (left side is the RFB and right side is the 5S rDNA) are the same as those of the wild type copy number strain. The IGS1 region was divided into seven segments, and each of them was replaced by *URA3* in a *FOB1* defective background. By reintroduction of *FOB1* gene, it was found that deletions of C–F (EXP) completely abolished the amplification ability of the repeats. EXP (~520 bp) contains the RFB, and E-pro (a non-coding bi-directional Pol II promoter).

the mechanism is not elucidated yet.

***Analysis of cis-acting factors.*** As the rDNA is a multi-copy gene region, it is almost impossible to mutate a part of an rDNA unit in all of the 150 rDNA units to check for amplification ability. Therefore determination of *cis*-acting elements for rDNA amplification is not as easy as that of *cis*-acting elements in other part of genome. To solve this problem, we constructed a strain in which the majority of rDNA repeats are deleted, leaving two copies of the rDNA (Fig. 3). In addition, *FOB1* needs to be disrupted in this strain to prevent amplification and maintain the two-copy situation. This strain can then be used to identify elements that are required for amplification. To do this, the target region is replaced with a *URA3* marker, and a plasmid-born *FOB1* gene is reintroduced to see whether the mutated rDNA unit can amplify or not. The IGS1 region, including the RFB/Fob1p recombinational hotspot, was analyzed by dividing it into seven segments (A–G, Fig. 3B). We found that replacement of four of these segments (C–F, ~520 bp) completely abolished amplification ability, therefore we named this *cis*-essential sequence for amplification the EXP (expansion of rDNA repeats, Kobayashi et al., 2001). The EXP contained the RFB as we expected.

By using the same strategy we found that the *rARS*, that is functioning as a replication origin, is also required for rDNA amplification (Kobayashi et al., in preparation). Therefore, this supports the model shown in Fig. 2, in which the rightward replication fork (Fig. 2-b) that is inhibited by the RFB site and induces DSB, is necessary for amplification.

**Non-coding transcription activates rDNA amplification by cohesin dissociation**

Although the EXP contains the RFB as discussed, still three quarters of the EXP sequence is independent of RFB activity (Kobayashi et al., 2001). The question is what role does this other part of the EXP play? In a recent phylogenetic footprinting study using various *Saccharomyces* species, we found a highly conserved sequence that corresponds to a previously-identified bi-directional Pol II promoter (Santangelo et al., 1988) in the EXP (Ganley et al., 2005, Fig. 3B). This EXP promoter (named E-pro) does not appear to be associated with any coding function, and its position and conservation suggested that it might play a role in rDNA gene amplification. We found that another bi-directional Pol II promoter (*GAL1/10*) could replace E-pro while unidirectional promoter were not able to replace it, thereby indicating that the transcription activity of E-pro has a critical role in rDNA amplification (Kobayashi and Ganley, 2005). This is the first finding of functional non-coding transcription in *S. cerevisiae*. In terms of the mechanism by which E-pro transcription induces the amplification, we found that cohesin dissociation caused by transcription is critical (Kobayashi and Ganley, 2005). Using an inducible E-pro promoter, we showed that repression of transcription allowed cohesin to associate with the rDNA, while activation of transcription removed this cohesin association. Cohesin is a multi-functional complex involved in chromatin structure, and it connects the sister-chromatids until they separate in M phase (Haering and Nasmyth, 2003). It is known that its localization is inversely-correlated with regions of transcription (Glynn, 2004; Lengronne, 2004), suggesting that transcription can physically remove cohesin from the chromosome. In addition, it was previously suggested that cohesin association holds chromatids in place, leading to equal sister-chromatid recombination and thereby preventing copy number change after DSB formation

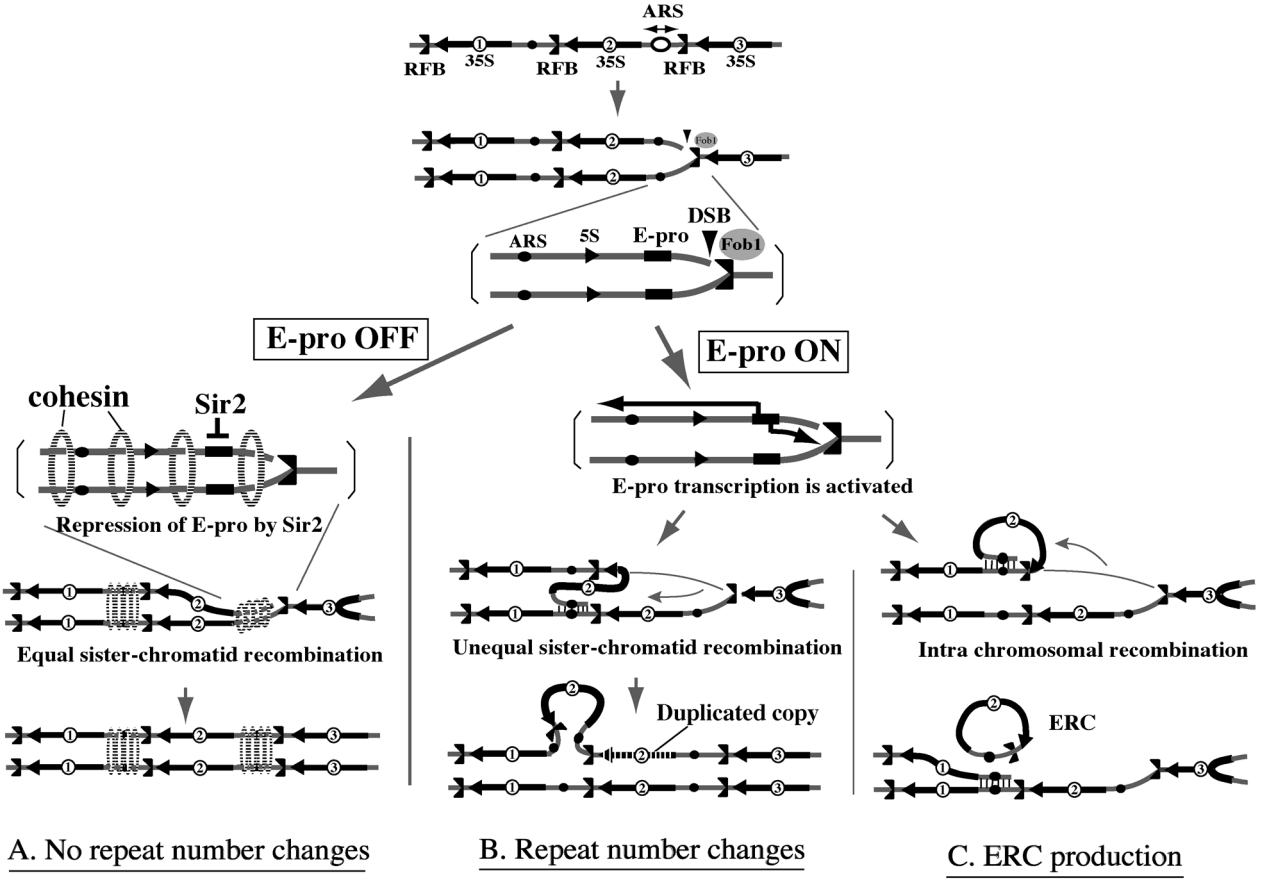


Fig. 4. The transcription-induced cohesin dissociation model of rDNA amplification. (**A**) In normal situations, such as wild-type rDNA copy number, Sir2p represses E-pro activity, allowing cohesin to associate with the IGS. DSBs are repaired by equal sister chromatid recombination, with no change in rDNA copy number. (**B**) In situations where Sir2p repression is removed, such as a *sir2* mutation, E-pro is activated and this transcription displaces cohesin from the IGS. This lack of cohesion means that unequal sister chromatids can be used as templates for repair of DSBs, resulting in changes in rDNA copy number as shown in Fig. 2. (C) In the same situation as (B), DSBs may be repaired by intra-chromosomal recombination. In this case, an ERC (extra rDNA circle) is popped out. The Lines represent single chromatids (double-strand DNA). The IGS in which the replication fork is paused is expanded in the bracket.

(Kobayashi et al., 2004). Therefore, dissociation of cohesin by E-pro transcription likely facilitates the broken end during DSB repair to move into a non-equal rDNA unit, leading to unequal sister-chromatid recombination, as shown in Fig. 4B (Kobayashi and Ganley, 2005). This cohesin dissociation also can induce intra-sister chromosomal recombination (Fig. 4C). This recombination produces extra-chromosomal rDNA circles (ERC) whose accumulation is thought to be a factor to accelerate cellular aging (Sinclair and Guarente, 1997).

***SIR2* is a negative regulator of E-pro transcription**

E-pro transcription was found to be a critical factor for the amplification through cohesin dissociation, however the question then becomes is how is this transcription is regulated? One possible candidate is the silencing protein, Sir2p. Sir2p is a NAD-dependent histone deacetylase that can alter chromatin structure (Imai et al., 2000; Fritze et al., 1997). It is known that *SIR2*-dependent silencing represses transcription of Pol II genes that have been integrated into the rDNA (Smith and Boeke, 1997; Bryk et al., 1997) and this gene function is required for rDNA stability (Gottlieb and Esposito, 1989). Moreover, *SIR2* was also shown to repress rDNA copy number change through effects on cohesin association (Kobayashi et al., 2004). Indeed, when we tested the possibility that the transcription level of E-pro is repressed by the presence of *SIR2*, we found this was the case (Kobayashi and Ganley, 2005). In addition, in an E-pro-less strain loss of *SIR2* function did not affect the rDNA stability, in contrast to a wild type strain where the rDNA is highly unstable when *SIR2* function is lost. Therefore, E-pro seems to be a major target of Sir2p, leading to rDNA stability (Kobayashi and Ganley, 2005, Fig. 4A). Regulation of amplification can therefore be achieved by turning off and on Sir2p association with E-pro.

**Condensin is important for rDNA stability in M phase**

Condensin is a protein complex that promotes chromatin condensation in M phase to facilitate segregation (for review, see Hirano, 2000). In *S. cerevisiae*, condensation is not obvious cytologically compared to the situation in higher eukaryotic cells, except in the rDNA region (Freeman et al., 2000). In yeast condensin is localized in the nucleolus (Freeman et al., 2000) and temperature sensitive (ts) mutations are known to increase rDNA instability more than 60 times over the wild type level, as measured by marker loss frequency (Bhalla et al., 2002), therefore a major target of condensin in *S. cerevisiae* is thought to be the rDNA. It has actually been observed under microscope that in a condensation defective mutant (*cdc14*), the rDNA region is decondensed and presumably entangled, and this seems to prevent the proper separation of the sister chromosomes in anaphase (D'Amours et al., 2004: Sullivan et al., 2004). It was reported that there is a peak of condensin association around the RFB region (Freeman et al., 2000: Johzuka et al., 2006), and also in non-rDNA regions there is a tendency that replication terminus regions associate with condensin (Wang at al., 2005). These suggest that condensin may be related to the torsional stress (utilizing or releasing) that is produced by the encounter of two replication forks to start condensing chromosomes after replication has finished. We will have to wait for further analysis to understand how this condensation occurs.

## DISCUSSION

In this review we have discussed the evidence that rDNA stability is maintained by collaboration of recombination, cohesion, and condensation. Recombination (in the form of amplification) increases copy number after deletion of repeats, cohesion leads to equal sister-chromatid recombination by holding broken sister-chromatid ends in place, and condensation of rDNA makes proper segregation of the region possible in M phase. The reason that the rDNA repeats require such careful handling is that they are one of the most fragile sites in the genome. This in turn suggests that if the rDNA is maintained stably, overall genome stability will be concomitantly increased. There is evidence that rDNA instability enhances cell senescence in yeast. Loss of *SIR2* function not only increases rDNA instability, it also shortens the lifespan to about half of that of the wild type (Kaeberlein et al., 1999). In contrast, a mutation of *FOB1* represses rDNA recombination and extends the lifespan by more than 50% (Defossez et al., 1999; Takeuchi et al., 2003). These findings support the idea that the rDNA is a fragile site, and by increasing the stability of the rDNA, the stability of the genome as a whole is increased, therefore affecting the lifespan. As described above, ERC accumulation also has a negative effect on lifespan, although the mechanism is still unknown. It is possible that rDNA instability may be triggering secondary events that are then responsible for reducing lifespan. For instance, as rDNA instability increases, more repair enzymes may concentrate at this locus, and therefore non-rDNA stability may be reduced. In addition, high levels of rDNA instability may induce cell cycle arrest for extended periods through checkpoint controls, thereby disrupting cell function.

Finally, turning the viewpoint to the positive effects of rDNA instability, the rDNA may have a function as a primary sensor of genome damage. When the genome is exposed to exogeneous (*e.g.* UV) or endogeneous (*e.g.* NTP analogues) DNA damaging factors, the rDNA is highly sensitive to this damage. It quickly triggers cell cycle arrest through checkpoint controls, and following this the damage can be repaired. We know that less than 50% of the rDNA repeat units are transcribed (Dammann et al.,

1995), therefore it is possible that one role of these excess copies is to play such kinds of extra-coding functions. Further study will be required to elucidate precisely what these functions are.

I thank Dr. A. R. D. Ganley of our laboratory for critical reading and preparation of the manuscript. This work was supported by grants 13141205, 17080010, and 17370065 from the Ministry of Education, Science and Culture, Japan, and by a Human Frontier Science Program grant.

## REFERENCES

Bhalla, N., Biggins, S., and Murray, A. W. (2002) Mutation of *YCS4*, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior. Mol. Biol. Cell. **13**, 632–645.

Brewer, B. J., and Fangman, W. L. (1991) Mapping replication origins in yeast chromosomes. Bioessays **13**, 317–322.

Brewer, B. J., Lockshon, D., and Fangman, W. L. (1992) The arrest of replication forks in the rDNA of yeast occurs independently of transcription. Cell **71**, 267–276.

Bryk, M., Banerjee, M., Murphy, M., Knudsen, K. E., Garfinkel D. J., and Curcio, M. J. (1997) Transcriptional silencing of Ty1 elements in the *RDN1* locus of yeast. Genes Dev. **11**, 255–269.

Burkhalter, M. D., and Sogo, J. M. (2004) rDNA enhancer affects replication initiation and mitotic recombination: Fob1 mediates nucleolytic processing independently of replication. Mol. Cell **15**, 409–421.

Coelho, P. S., Bryan, A. C., Kumar, A., Shadel, G. S., and Snyder, M. (2002) A novel mitochondrial protein, Tar1p, is encoded on the antisense strand of the nuclear 25S rDNA. Genes Dev. **16**, 2755–2760.

Dammann, R., Lucchini, R., Koller, T., and Sogo, J. M. (1995) Transcription in the yeast rRNA gene locus: distribution of the active gene copies and chromatin structure of their flanking regulatory sequences. Mol. Cell. Biol. **15**, 5294–5303.

D'Amours, D., Stegmeier, F., and Amon, A. (2004) Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. Cell **117**, 455–469.

Defossez, P. A., Prusty, R., Kaeberlein, M., Lin, S. J., Ferrigno, P., Silver, P. A., Keil, R. L., and Guarente, L. (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell **3**, 447–455.

Ellwood, M., and Nomura, M. (1982) Chromosomal locations of the genes for rRNA in *Escherichia coli* K-12. J. Bacteriol. **149**, 458–468.

Freeman, L., Aragon-Alcaide, L., and Strunnikov, A. (2000) The condensin complex governs chromosome condensation and mitotic transmission of rDNA. J. Cell Biol. **149**, 811–824.

Fritze, C. E., Verschueren, K., Strich, R., and Easton, E. R. (1997) Direct evidence for *SIR2* modulation of chromatin structure in yeast rDNA. EMBO J. **16**, 6495–6509.

Ganley, A. R. D., 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.

Glynn, E. F., Megee, P. C., Yu, H. G., Mistrot, C., Unal, E., Koshland, D. E., DeRisi, J. L., and Gerton, J. L. (2004) Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. PLoS Biol. **2**, E259.

Gottlieb, S., and Esposito, R. E. (1989) A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. Cell **56**, 771–776.

Haering, C. H., and Nasmyth, K. (2003) Building and breaking bridges between sister chromatids. BioEssays **25**, 1178–1191.

Hirano, T. (2000) Chromosome cohesion, condensation and separation. Annu. Rev. Biochem., **69**, 115–144.

Huang, J., and Moazed, D. (2003) Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. Genes Dev. **17**, 2162–2176.

Imai, S., Armstrong, C. M., Kaeberlein, M., and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD- dependent histone deacetylase. Nature **403**, 795–800.

Ingle, J., Timmis, J. N. and Sinclair, J., (1975) The Relationship between Satellite Deoxyribonucleic Acid, Ribosomal Ribonucleic Acid Gene Redundancy, and Genome Size in Plants. Plant Physiol. **55**, 496–501

Johzuka, K., Terasawa, M., Ogawa, H., Ogawa, T., and Horiuchi, T. (2006) Condensin loaded onto the replication fork barrier site in the rRNA gene repeats during S phase in a *FOB1*-dependent fashion to prevent contraction of a long repetitive array in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **26**, 2226–2236.

Kaeberlein, M., McVey, M., and Guarente, L. (1999) The *SIR2/3/4* complex and *SIR2* alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. **13**, 2570–2580.

Kobayashi, T. (2003) The replication fork barrier site forms a unique structure with Fob1p and inhibits the replication fork. Mol. Cell. Biol. **23**, 9178–9188.

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

Kobayashi, T., Heck, D. J., Nomura, M., and Horiuchi, T. (1998) Expansion and contraction of ribosomal DNA repeats in Saccharomyces cerevisiae: requirement of replication fork blocking (Fob1) protein and the role of RNA polymerase I. Genes Dev. **12**, 3821–3830.

Kobayashi, T., Hidaka, M., Nishizawa, M., and Horiuchi, T. (1992) Identification of a site required for DNA replication fork blocking activity in the rRNA gene cluster in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **233**, 355–362.

Kobayashi, T., Horiuchi, T., Tongaonkar, P., Vu, L., and Nomura, M. (2004) *SIR2* regulates recombination between different rDNA repeats, but not recombination within individual rRNA genes in yeast. Cell **117**, 441–453.

Kobayashi, T., Nomura, M., and Horiuch, T. (2001) Identification of DNA *cis* elements essential for expansion of ribosomal DNA repeats in *Saccharomyces cerevisiae*: Mol. Cell. Biol. **21**, 136–147.

Laloraya, S., Guacci, V., and Koshland, D. (2000) Chromosomal addresses of the cohesin component Mcd1p. J. Cell Biol. **151**, 1047–1056.

Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G. P., Itoh, T., Watanabe, Y., Shirahige, K., and Uhlmann, F. (2004) Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature, **430**, 573–578.

Long, E. O., and Dawid, I. B. (1980) Repeated genes in eukaryotes. Annu. Rev. Biochem. **49**, 727–764.

Pruitt, R. E., and Meyerowitz, E. M. (1986) Characterization of the genome of *Arabidopsis thaliana*. J. Mol. Biol. **187**, 169–183.

Rodland, K. D., and Russell, P. J. (1982) Regulation of ribosomal RNA cistron number in a strain of Neurospora crassa with a duplication of the nucleolus organizer region. Biochim. Biophys. Acta. **697**, 162–169.

Sakai, K., Ohta, T., Minoshima, S., Kudoh, J., Wang, Y., de Jong. P. J., and Shimizu, N. (1995) Human ribosomal RNA gene cluster: identification of the proximal end containing a novel tandem repeat sequence. Genomics, **26**, 521–526.

Santangelo, G. M., Tornow, J., McLaughlin, C. S., and Moldave, K. (1988) Properties of promoters cloned randomly from the *Saccharomyces cerevisiae* genome. Mol. Cell. Biol. **8**, 4217–4224.

Sinclair, D. A., and Guarente, L. (1997) Extrachromosomal rDNA circles-a cause of aging in yeast. Cell **91**, 1033–1042.

Smith, J. S., and Boeke, J. D. (1997) An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. **11,** 241–254.

Sullivan, M., Higuchi, T., Katis, V. L., and Uhlmann, F. (2004) Cdc14 phosphatase induces rDNA condensation and resolves cohesin-independent cohesion during budding yeast anaphase. Cell **117**, 471–482.

Takeuchi, Y., Horiuch, T., and Kobayashi, T. (2003) Transcription-dependent recombination and the role of fork collision in yeast rDNA. Genes Dev. **17**, 1497–1506.

Tartof, K. D. (1979). Evolution of transcribed and spacer sequences in the ribosomal RNA genes of Drosophila. Cell **17**, 607–614.

Wallace, H., and Birnstiel, M. L. (1966) Ribosomal cistrons and the nucleolar organizer. Biochim. Biophys. Acta. **114**, 296–310.

Wang, B. D., Eyre, D., Basrai, M., Lichten, M., and Strunnikov, A. (2005) Condensin binding at distinct and specific chromosomal sites in the *Saccharomyces cerevisiae* genome. Mol. Cell. Biol. **25**, 7216–7225.

Weitao, T., Budd, M., Hoopes, L. L., and Campbell, J. L. (2003) Dna2 helicase/nuclease causes replicative fork stalling and double-strand breaks in the ribosomal DNA of *Saccharomyces cerevisiae*. J. Biol. Chem. **278**, 22513–22522.