DIVISION OF GENOME DYNAMICS

Professor
HORIUCHI, Takashi

Assistant Professors	JOHZUKA, Katsuki
	WATANABE, Taka-aki
Technical Staff	MOROOKA, Naoki
Postdoctoral Fellow	KODAMA, Ken-ichi
Graduate Students	ITAZU, Masako
	OKAMOTO, Haruko
Secretary	MIKAMI, Yuriko

The genomes of higher organisms contain significant amounts of repetitive sequences which are, in general, unstable. At present, neither the physiological function(s) of repeated sequence or the mechanisms producing repeated sequences and controlling instability are fully understood. To clarify these aspects of genomes, we are pursuing several themes using *Eschericia coli*, *Saccharomyces cerevisiae* and Chinese Hamster Ovary (CHO) cells. In 2007 we have made advances in understanding a new role for condensin in maintaining a long repeated structure of rDNA in yeast and a relationship between the association of condensin with chromosomes and transcription on those chromosomes. In addition, we also have constructed a new system of gene amplification via DRCR (double rolling circle replication) in yeast by using the Cre-*lox* site-specific recombination. From previous and present results, we concluded that DRCR is an actual amplification mechanism, at least in budding yeast. There exists a possibility that DRCR might work for gene amplification in higher eukaryotes as well.

Ⅰ. Analysis of mechanism maintaining repeated structure of ribosomal RNA genes

In most eukaryotic organisms, the rDNAs are clustered in long tandem repeats on one or a few chromosomes. Although the total number of these 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 a system to regulate recombination within rDNA repeat.

In the yeast *Saccharomyces cerevisiae*, 200 copies on average are tandemly arrayed in a central position on the longest 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 replication fork blocking activity at the replication fork barrier (RFB) site, rDNA region-specific recombination and expansion/contraction of rDNA repeats. The latter two activities are likely to be triggered by a double-strand break at the RFB site and a repairing of the break via gene conversion. Thus, this *FOB1*-dependent recombination apparently contributes to the maintenance of the average copy number of rDNA. However, in *Δfob1* cells, the repeats are still maintained without any fluctuation of copy number, thereby suggesting that another, unknown system acts to prevent contraction of the number of repeats.

In order to understand this putative second system, we collected a number of mutants in which the copy number of rDNA decreased drastically under *Δfob1* conditions. Among these, we found condensin defective mutants, suggesting that, in addition to the condensation and separation of chromosomes in M phase, condensin plays an important role in maintaining the repeated structure of rDNA. Each gene encoding condensin subunit is known to be essential for growth, but here isolated condensin mutation is not strict but leaky type. Analyzing the double mutants and examining the specific interaction between condensin and rDNA region revealed that (1) in the double mutants, the copy number of rDNA in the mutant dramatically decreased, (2) condensin complex associated with the RFB region in *FOB1*-dependent manner, (3) the association between condensin and RFB was established during S phase and was maintained until anaphase, and (4) double mutant showed slow growth which may be caused by a defect in the separation step of the long rDNA array in anaphase. These results strongly suggest that *FOB1*-dependent condensin association with RFB region is required for efficient segregation of rDNA repeated region.

Recently we found that RNA polymerase I (PolI) defective mutation suppresses dramatic reduction of the copy number of rDNA in the condensin and *fob1* double mutant. Because PolI is an rDNA specific transcription enzyme, ΔpolI defective mutant is non-viable. But if the defective cells carry a plasmid, in which rDNA is inserted downstream of Gal-dependent promoter, cells can be viable in the presence of galactose. Using the ΔpolI mutant, we examined the effect of PolI enzyme on condensin association with rDNA. Under *fob1* conditions, PolI enzyme seems to force condensin from transcribing to non-transcribing (IGS) region. This characteristic change of pattern suggests that constant PolI transcription through cell cycle prevents condensin from associating with the transcribing region. Thus, it is expected that, in the triple (*fob1*, condensin and PolI) mutant, the partial defective condensin uniformly associated with rDNA region is responsible for successful separation in anaphase.

In higher eucaryotes, it is well known that M phase-specific repression of gene expression (mitotic repression) occurs, though the reason remains unknown. Our results suggest that the mitotic repression allows condensin to associate uniformly with whole chromosomes that ensure their successful condensation and subsequent separation. At any rate, genetic and molecular analyses of highly specific chromosomal regions like rDNA provide useful data which help to understand the nature of normal (non-rDNA) chromosomal regions.

Ⅱ. Mechanism of condensin recruitment onto RFB site located within the tandem rDNA repeat in budding yeast.

The primary functions of mitotic chromosome condensation are to reduce the length of chromosomes so

that they avoid truncation of genome during cell division and to ensure the proper segregation of sister chromatids. The compaction ratio of mitotic chromosomes relative to double stranded DNA fiber ranges from ~160-fold in budding yeast to ~10000 – 20000-fold in mammalian chromosomes. Condensin is a multi-subunit protein complex that plays a central role in mitotic chromosome condensation and segregation. In vertebrates, condensin has been shown to be distributed in axial part over the whole length of condensed chromosomes, but only at the resolution of light microscopy. The sites where condensin acts in chromatin and the molecular mechanisms of condensin recruitment have largely remained elusive. As described above, we found that condensin localized at the RFB site in Fob1 dependent manner during S-phase. To date, this Fob1-dependent condensin localization is the only example of condensin association with a specific DNA site in a specific protein factor-dependent manner. To understand chromosome condensation in molecular resolution, we are studying mechanisms of condensin localization at the RFB site. Firstly, we haven't obtained any positive data indicating direct interaction between condensin and Fob1 protein from either of two-hybrid or immuno-precipitation experiment, and we therefore excluded the possibility in Figure 1 (1). Secondly, we discovered that condensin could bind to the short DNA fragment containing RFB sequences, even if the sequence was inserted in either orientation (fork blocking or non-blocking orientation) at an ectopic chromosome site, and we therefore excluded the possibility in Figure 1 (2). To gain further information about the specific recruitment of condensin onto the RFB site, we isolated additional factors by genetic approach. So far, we have identified at least three additional factors that were necessary for condensin recruitment to the RFB site (Figure 1 (3)). Protein-protein interaction analysis of these three factors in addition to Fob1 and all subunits of condensin complex suggested that condensin was targeted at the RFB site by ordered interactions among them.

Ⅲ. Construction of a new gene amplification system via DRCR (double rolling circle replication) by using the Cre-*lox* site specific recombination

In addition to rDNA gene amplification in eukaryotes, there is another type of gene amplification, which 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. Previously, 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 two types of amplification products. Type-1 products contain 13 to ~100 copies of the amplification marker, *leu2d* (up to ~730 kb increase) with novel arrangement present as randomly oriented sequences flanked by inverted *leu2d* copies. Type-2

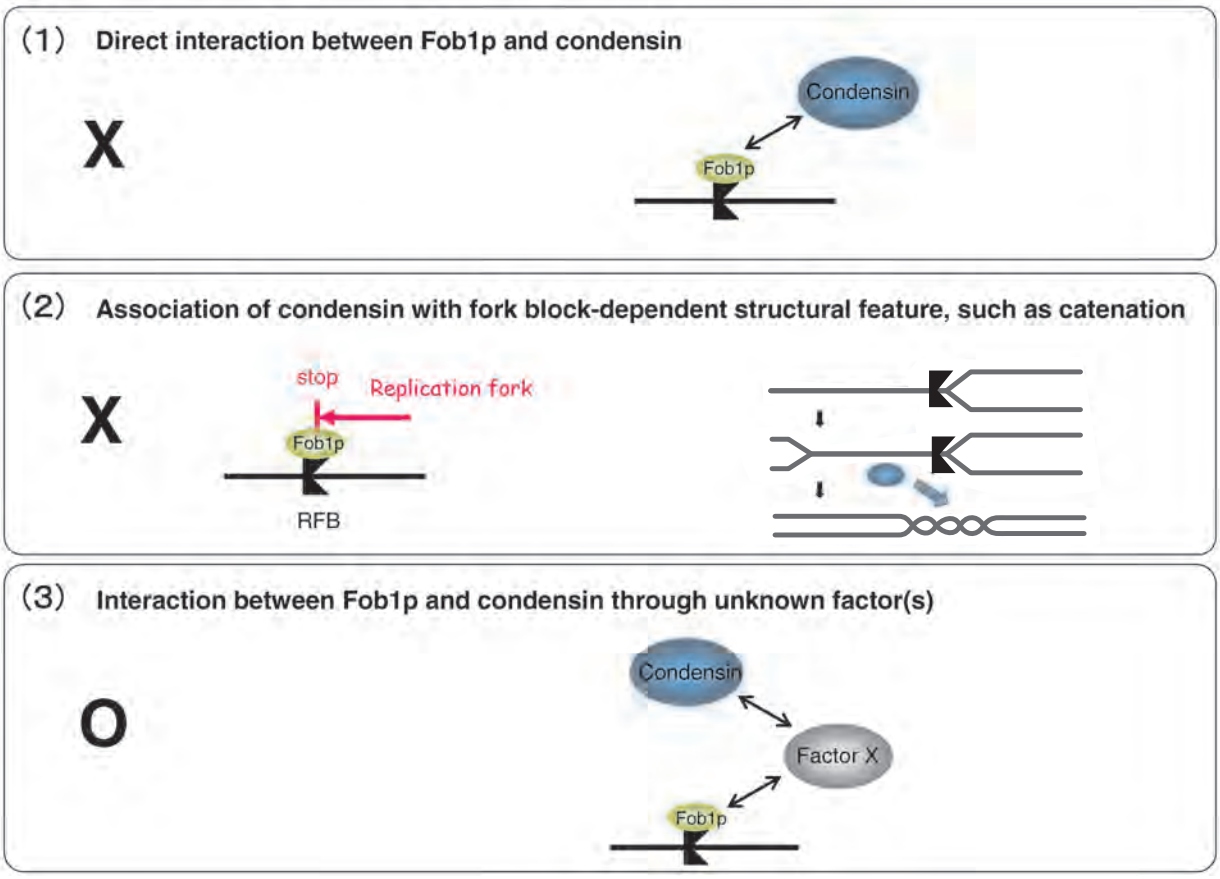

Figure 1. How to associate condensin with the specific RFB site in rDNA repeats

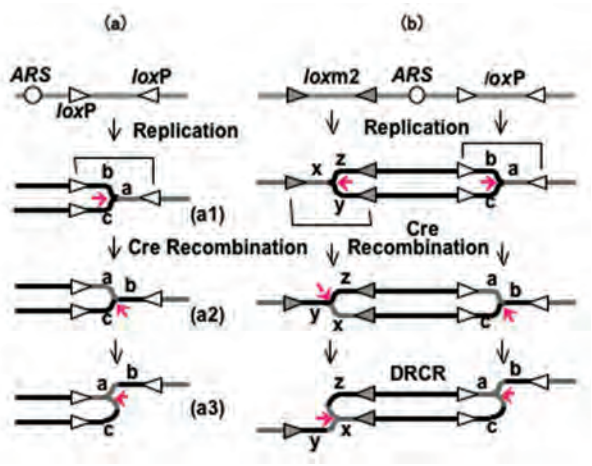

Figure 2. DRCR induced by Cre-*lox* system
(a) Cre-*lox* dependent reversal of replication orientation. (a1) When the replication fork passes through between a pair of *lox* sites, Cre recombination occurs between a pair of *lox* sites, as shown in (a1). Recombination changes replication orientation from un-replicated DNA (parental DNA strand) to replicated DNA (one of sister chromatids) as shown in (a2 and a3), because DNA strand a and b are identical. (b) DRCR is induced by Cre-dependent combinational recombination of two pairs of *lox*P and *lox*m2 as shown in (b). Recombination cannot occur between *lox*P and *lox*m2.

products are acentric multi-copy mini-chromosomes carrying *leu2d*. Structures of type-1 and -2 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 (*EMBO J* 24, 190-198 (2005)).

If DRCR is an actual gene amplification mechanism in yeast, a quite different initiation reaction, which can induce RDCR, should produce amplification products resembling to HSR and DMs. Thus, we tried to construct a new DRCR amplification system that is induced by another process, Cre-*lox* site-specific recombination. We first predicted that, if Cre recombination occurs between the two *lox* sites, one present on the replicated and the other on the unreplicated regions, as shown in Figure 2(a1), the replication fork should switch the template from the parental (unreplicated) to the sister-chromatid (replicated) DNA strands, as shown in Figure 2(a2) and (a3), and that the Cre recombination system would make this process efficient, as shown in Figure 2(a). Furthermore, a combination of the process, as shown in Figure 2(b), can efficiently induce gene amplification through DRCR. In actuality, this system produced two kinds of products; highly amplified (>100 copies) chromosome products and acentric multi-copy extra-chromosomal products. The structures of these products resemble those of HSR and DMs of higher eukaryotes, respectively. From previous and present results, we concluded that DRCR is an actual amplification mechanism in budding yeast and could be naturally initiated if some structural requirement could be satisfied.

Publication List

〔Original papers〕

- Cui, T., Moro-oka, N., Ohsumi, K., Kodama, K., Ohshima, T., Ogasawara, N., Mori, H., Wanner, B., Niki, H., and Horiuchi, T. (2007). *E. coli* with a linear genome. EMBO Rep. *8*, 181-187.
- Ganley, A.R., and Kobayashi, T. (2007). Highly efficient concerted evolution in the ribosomal DNA repeats: total rDNA repeat variation revealed by whole-genome shotgun sequence data. Genome Research *17*, 184-191.
- Johzuka, K., and Horiuchi, T. (2007). RNA polymerase I transcription obstructs condensin association with 35S rRNA coding region and can cause contraction of long repeat in *Saccharomyces cerevisiae*. Genes Cells *12*, 759-771.