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The genomes of higher organisms contain significant amounts of repetitive sequences which, in general, are unstable. At present, neither the physiological function(s) of these repeated sequences, nor the mechanism producing repeated sequences and controlling instability are fully understood. To clarify these aspects, we are pursuing several themes using *Saccharomyces cerevisiae*, and Chinese Hamster Ovary (CHO) cells. In 2009 we have made progress in understanding a new role of condensin in maintaining the long repeated structure of rDNA, in which it is an essential step that condensin is recruited to the *RFB* (replication fork barrier) site in rDNA repeat units by three protein factors (Tof2p, Csm1p and Lrs4p) and Fob1 protein. In addition, we also constructed a new system of gene amplification via DRCR (double rolling circle replication) in yeast by using Cre-*lox* site-specific recombination. Furthermore we constructed an analogous system for the CHO chromosome and found that it produced gene amplification products also seen in tissue cultured cells under natural conditions.

Previously we constructed *Eschericia coli* cells with a linear genome and found they behave like *E. coli* with a circular genome (Cui *et al*., EMBO Rep. *8*, 181-187, 2007). We investigated how the genome behaves when the circular genome is linearized by TelN protein.

I. Mechanism of condensin recruitment onto the RFB site located within rDNA repeats in budding yeast

The primary functions of chromosome condensation during cell division are to facilitate the individualization of sister chromatids, which remain entangled after DNA replication, and to shorten the length of chromatid arms so that they avoid truncation during chromosome segregation to opposite poles of the cell. The compaction ratio of mitotic chromosomes relative to double stranded DNA ranges from ~160-fold in budding yeast to ~10000-20000-fold in mammalian chromosomes. Despite its being a fundamental process for cell growth, we have a surprisingly poor understanding of the higher levels of chromosome organization. Many textbooks feature the radial loop model of condensed mitotic chromosomes in which the chromatin fibers are bunched around a chromosome scaffold; this model is largely based on electron microscopy of mitotic chromosomes under high-salt or polyanion conditions. One clue for understanding the structure of condensed chromosomes was the identification of components of the chromosomal scaffold, namely, Top2 and condensin. Although, both of these are known to be required for building up condensed mitotic chromosomes, their relationship to specific DNA elements is still poorly understood. Condensin is a multi-subunit protein complex that plays a central role in mitotic chromosome condensation and segregation. In vertebrates, condensin is distributed in the axial part over the whole length of condensed chromosomes, but this has only been shown at the resolution of light microscopy. The sites where condensin acts on the chromatin and the molecular mechanisms of condensin recruitment onto thereto had largely remained elusive. In previous studies, we found that condensin localized to the *RFB* site in a Fob1 (fork block protein) dependent manner during S-phage. To date, this Fob1p-dependent condensin localization is the only example of condensin association with a specific DNA site in a specific protein factor-dependent manner. With the goal of understanding chromosome condensation at the molecular level, we have studied mechanisms of condensin localization at the *RFB* site. This year we discovered that condensin can bind to a short DNA fragment containing *RFB* sequences, even if the sequence was inserted at an ectopic chromosomal site. This indicates that the *RFB* site itself acts as a site recruiting onto chromatin. Analysis of the relationship between condensin recruitment to the *RFB* site and Fob1p-dependent replication fork blockage at the *RFB* site demonstrated that those two events were completely independent phenomena. Instead, we identified three additional protein factors, Tof2p, Csm1p, and Lrs4p, necessary for both *FOB1*-dependent condensin recruitment to the *RFB* site and for ensuring the faithful segregation of long rDNA repeats. We also found ordered binding of Fob1p, Tof2p, Csm1p/Lrs4p, and condensin complex at the *RFB* site, as shown in Figure 1. Finally, *in vivo* interaction between Csm1p, Lrs4p and multiple subunits of condensin were detected. These results suggest that condensin is recruited to the *RFB* site by the sequential interaction of Fob1p, Tof2p, Csm1p, Lrs4p, and finally condensin, to ensure the proper segregation of long rDNA tandem array.

II. Condensin contributes to repressing transcriptions by RNA polymerase II in the rDNA cluster

As described above, we found that the replication fork barrier (*RFB*) site, which is located in the intergenic spacer region (IGS) within the ribosomal RNA gene (rDNA) cluster, worked as a cis element for condensin recruitment to chromatin. Interestingly, recruitment of condensin to the RFB site occurs in the DNA synthesis-phase, long before mitotic-phase, suggesting that condensin plays some role during interphase. We are investigating the mechanism of condensin recruitment to chromatin and its role in maintaining the integrity and the higher order structure of the rDNA repeated region. This year, we discovered that condensin contributed to silencing of RNA polymerase II

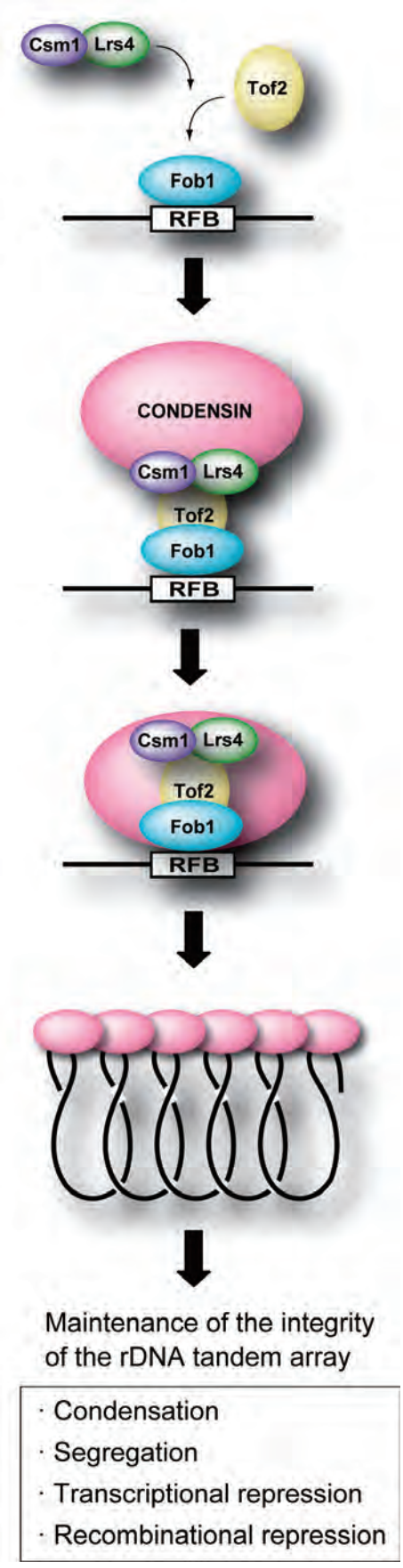

Figure 1. Model for condensin recruitment to the *RFB* site, contributing to maintenance of the integrity of long rDNA repeats.

transcription from a reporter gene inserted close to the RFB site. Condensin mutation leads to a decrease in the Sir2 association profile in rDNA. As well as the changes in the Sir2 association profile, endogenous transcriptions by RNA polymerase II within the IGS region increased in condensin mutants. Based on these observations, we hypothesize that condensin has an effect on the higher-order chromatin structures of the IGS region, resulting in the promotion of Sir2 accessibility to the region, thus contributing to transcriptional silencing.

III. Construction of a new gene amplification system via DRCR (double rolling circle replication) using Cre-lox site specific recombination in budding yeast and CHO (Chinese hamster ovary) cell

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, *lue2d* (up to 730 kb increase) with novel arrangement present as randomly oriented sequences flanked by inverted *leu2d* copies. Type-2 products are acentric multi-copy mini-chromosomes, each carrying two *lue2d* copies. Structures of type-1 and -2 products resemble those of homogeneously staining regions (HSRs) and double minutes (DMs) of higher eukaryotes, respectively. Interestingly, products analogous to theses were generated at low frequency without deliberate DNA cleavage.

If DRCR were an actual gene amplification mechanism in yeast, a quite different initiation reaction, which can induce DRCR, should produce amplification products resembling HSRs and DMs. Thus, we tried to construct a new DRCR amplification system that is induced by another process; Cre-*lox* site-specific recombination. Cre-*lox* site-specific recombination occurs between un-replicated and replicated regions during replication, the fork replicates these already-replicated regions again, and the Cre recombination system makes this process more efficient. Furthermore, a combination of the process efficiently induces gene amplification through DRCR. In fact this system produced two kinds of products; the structures of these products resemble those of HSRs and DMs of higher eukaryotes, respectively. Furthermore, the same genetic elements produce HSR- and DM-like products when placed in cultured mammalian (CHO) cells. Thus, we concluded that DRCR is indeed an amplification mechanism in lower and higher eukaryotes and can be naturally initiated if some structural requirement is satisfied.

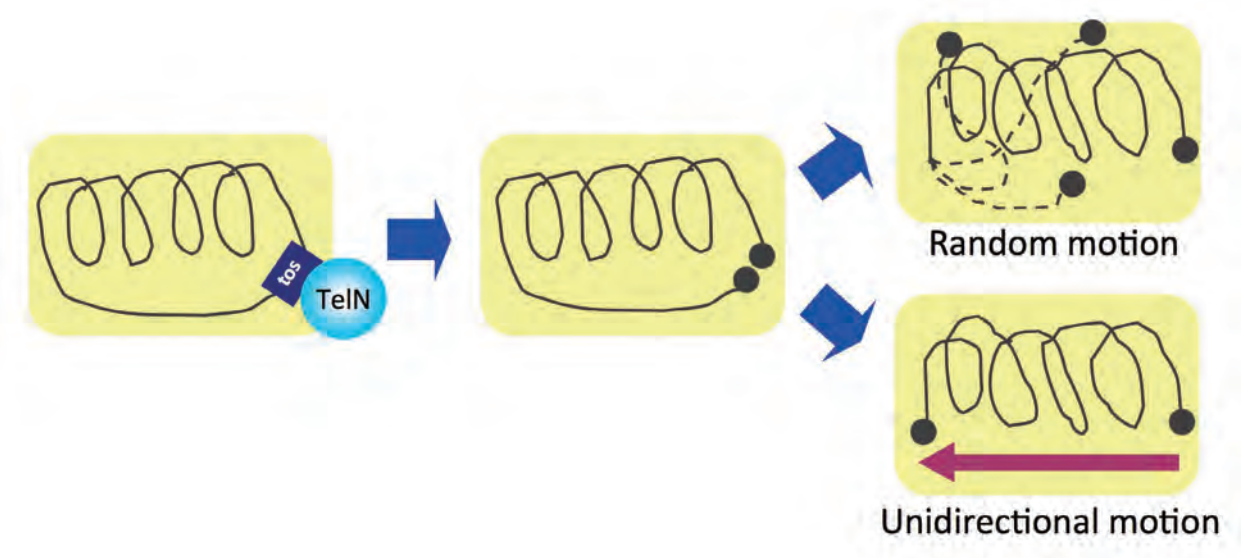

Figure 2. How do two termini of *E. coli* genome behave when the circular genome is linerized?

IV. *E. coli* with linear genome: how do two termini of the genome behave when the circular genome is linearized?

Previously, we constructed *E. coli* with a linear genome (Cui *et al*., EMBO Rep. *8*, 181-187, 2007). Interestingly, the *E. coli* strain with a linear genome did not show any distinct characteristics in comparison with circular genome *E. coli*. Here, we investigate how each end of the linear genome behaves when the circular genome is linerized with TelN enzyme (linearization enzyme coded in N15 lysogenic phage genome). We expected two possibilities; one was that each end would move randomly, at least for a while, the other was that each end would separate to each pole position of the cell (Figure 2).

In order to know which possibility was correct, the following *E. coli* strain was constructed; *tos* sequence, a target sequence for TelN enzyme, was inserted into the site within the replication terminus region (the site close to *dif*), an opposite region of replication origin (*oriC*) of the circular genome. Then, at both sides of *tos*, two kinds of operator (*tetO* and *lacO*) repeated sequences were inserted. Finally, CFP-*tet* and YFP-*lac* repressor genes were inserted downstream of arabinose promoter, and *telN* gene was positioned downstream of a rhamnose promoter on the *E. coli* genome.

The strain was grown in M9-succinate medium logalismic phase and then arabinose was added to the medium to induce CFP-*tet* and YFP-*lac* repressors, which bind to the *tetO* and *lacO* sites, respectively, close to each other before linearization. Then, by addition of rhamnose to the culture, TelN enzyme was induced, which cut the *tos* site and converted the circular genome to a linear one. During these experiments, we examined the behavior of two fluorescent spots under a microscope. The observations show that the two terminuses, after linearization, do not move randomly, but one stays in place and the other tends to move to the other pole site of the cell. Average rate of the latter type of movement is approximately 0.216μ/min. Because the DNA is neither dramatically stretched nor shrunk, these results strongly suggest participation of proteins.

Publication List

〔Original paper〕

- Johzuka, K., and Horiuchi, T. (2009). The *cis*-element and factors required for condensin recruitment to chromosome. Mol. Cell *34*, 26-35.