DIVISION OF GENE EXPRESSION AND REGULATION II

Professor: *Takashi Horiuchi*
Research Associate: *Masumi Hidaka*
Takehiko Kobayashi
Ken-ichi Kodama
**Katsuki Johzuka*
Technical Staff: *Kohji Hayashi*
Yasushi Takeuchi
Graduate Student: *Hiroko Urawa*

**to Department of Biological Chemistry, University of California-Irvine, Irvine, California (Feb.1. 1999-)*

Homologous recombination, which may occur in all organisms, apparently involves genetic exchange between two parent-derived chromatids in addition to the repair of DNA damage induced by physical and chemical reagents. As deduced from our analyses of recombinational hotspots of *E. coli* and *S. cerevisiae*, in particular the activity related to DNA replication fork blocking events, the physiological function of homologous recombination (especially in normally growing cells) is better understood. In 1999, work on the two following subjects has advanced our knowledge of both the dynamics and function of genome.

I. Replication fork block protein (Fob1) acts as a rDNA specific recombinator.

We previously found that some recombinational hotspots in *E. coli* are regions where progress of a DNA replication fork is severely impeded. Detailed analysis of this phenomenon revealed that when the fork is blocked, recombinational enzymes construct a new replication fork after breakdown of the arrested fork by introducing a double-stranded break at either of the sister chromosomes. Consequently, this recombination reaction for renewal of the replication fork is a primary cause resulting in recombinational hotspots.

Next, we showed that this phenomenon occurred not only in prokaryotes but also in eucaryotes. In most eucaryotes, the DNA replication fork blocking site, called *RFB* (replication fork barrier), is located in each unit of rRNA multi-genes (rDNA). It has been reported that in budding yeast (*S. cerevisiae*) when a non-transcriptional region (NTS) of the rDNA, in which *RFB* site is located, is inserted into a non-rDNA region of chromosomes, recombination of a region close to the insertion site is enhanced. This enhancement is called *HOT1* activity and the DNA fragment required for activation is named *HOT1* DNA. We isolated a number of mutants, which are defective in *HOT1* activity, and found that some of them had a concomitant defect in fork blocking activity at the *RFB* site. Both of these defective phenotypes were restored to wild type by introducing a single gene, named *FOB1*, thereby suggesting that fork blockage acts as a trigger for recombination in yeast, as well. In addition to this finding, the detection of *RFB* activity within the rDNA cluster in various higher eucaryotic cells indicates that the fork renewal cycle occurring after the blockage is more general than expected.

A copy number of rDNA repeats is unstable. Until recently, though an increase and a decrease in a number of rDNA copies have been observed in various eucaryotes, the underlying mechanism remains unknown. Because fork arrest at the *RFB* site was a trigger of recombination, as mentioned above, we suspected that a fork-blocking event was involved in changing the copy number. Upon examination it turned out that in a *fob1* mutant neither an increase nor a decrease in the rDNA copy number took place; thereby suggesting that the fork blocking event is required for changing the copy number, probably through recombination process. Since fork blocking system has only been understood to prevent replication from "colliding" with rDNA transcription so far, this study sheds light a novel physiological role for fork blocking system. The newly discovered role may be quite a common in most of eucaryotes.

This year we conducted a molecular examination as to whether the fork blocking step is actually essential for homologous recombination within the rDNA repeated region. In this case, recombination means intra- or inter-sister chromosomal exchange. Because the intra-sister chromosomal recombination between two repeats of the rDNA produces a covalently closed circular (ccc) molecule, its recombination activity can be assayed easily by measuring the quantity of ccc molecules produced. In the case of inter-chromosomal recombination, since the structure of the rDNA cluster remains unchanged whether inter-recombination occurs or not, the recombination product is hard to detect. However, because rDNA has a repeated structure, if any selective maker (*URA3* marker in this experiment) is inserted anywhere in the rDNA region, unequal inter-sister chromosomal recombination can be assayed by detecting duplicated molecules of the inserted marker. Using these two assay methods, we investigated whether *FOB1* or other recombination genes were involved in recombination of the rDNA region and the following results were obtained: (1) the frequency of intra- and inter-recombination in both *fob1* and *rad52* mutants decreased to one tenth of those in the wild type strain (Figure A), (2) while the *RAD52* gene was a general recombinator, the *FOB1* gene was a rDNA region-specific recombinator, (3) a major type of recombination was of the inter-sister chromosomal type, while intra-recombination was minor, (4) at least an inter-recombination occurred uniformly throughout the rDNA region, (5) enhancement of rDNA region-specific recombination by the *sir2* (silencer gene 2) mutation was found to be *FOB1*-dependent.

Guarente's group at MIT has reported that the life span of budding yeast is determined by the number of ccc molecule of rDNA accumulated in mother cells. ccc rDNA molecules, which is excised from the rDNA cluster on the chromosome, are not segregated equally into daughter and mother cells but are accumulated only

in the mother cells. Thus, mother cells "die" after about 20 generations. If the group's speculation is correct, the *fob1* mutant is expected to have a longer life span than the wild type strain. Actually, we found that the *fob1* mutant could live 60 % longer than the wild type strain (Figure B). Independently, Guarente's group also reported a similar result. These findings raised the possibility that the ccc rDNA molecule might also be involved in determining the life span of multicellular organisms. In order to investigate this possibility, isolation and analysis of their *FOB1*-like gene defective mutants should prove to be a successful approach.

II. *E. coli* genomic structure and function

A project named "Structure and function of the *E. coli* genome", supported by a grant termed CREST from JST for a duration of five years, began in January 1999. The team is composed of twelve independent research groups that are divided into four main categories of research. Namely, these divisions are: (1) resource, (2) bio-informatics, (3) database, and (4) systematic functional analysis. At present, we are determining the sequences of a remaining region (about 370 kb) of the genome of *E. coli* W3110 strain and will complete it until this summer. The resource group has constructed a large number of gene disruptants using a random transposon insertion method, in which the insertion sites will be determined by sequencing in part in this laboratory. Our immediate goal is to establish a systematic method for screening the phenotypes of about 4000 different disruptants, each of which has disrupted mutation in each of about 4000 nonessential genes.

Publication List:

Taki, K., Horiuchi, T
The SOS response is induced by replication fork blockage at a *Ter* site located on a pUC-derived plasmid: dependence on the distance between *ori* and *Ter* sites. Mol. Gen. Genet. (1999) 262, 302-309.

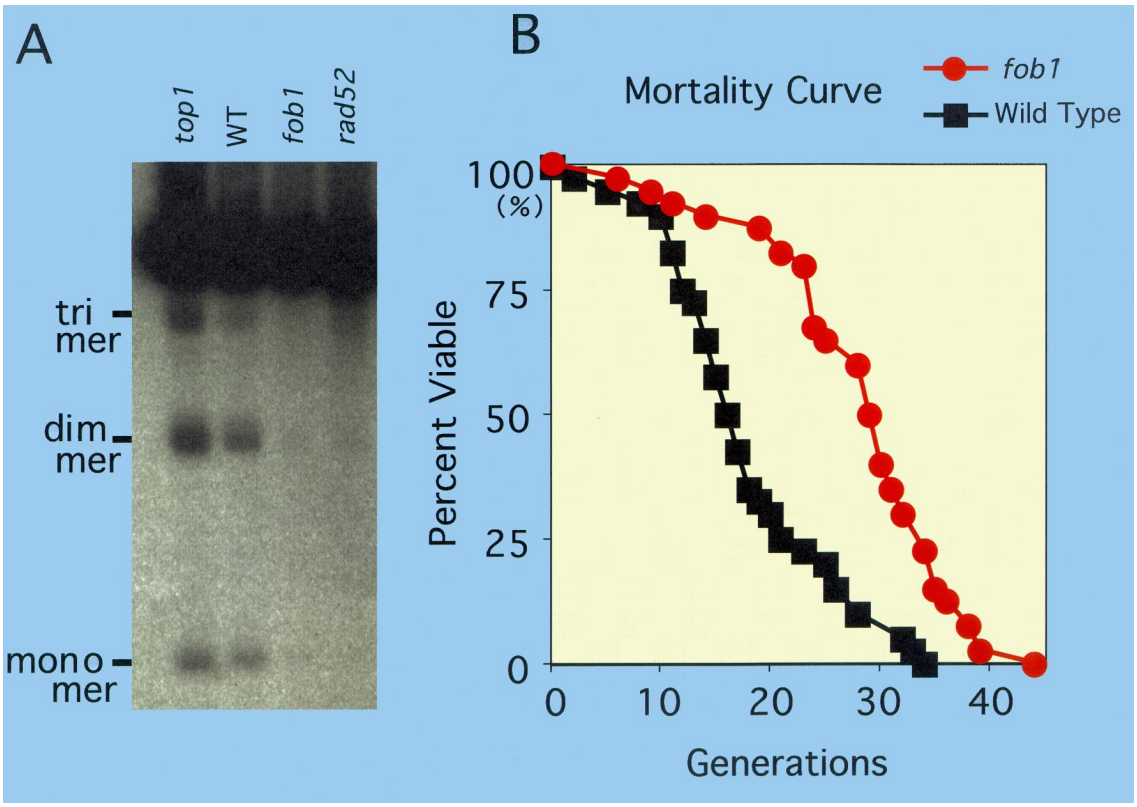

Figure: *fob1* is a long life mutant.
(A) Production of covalently closed circular (ccc) rDNA molecules (mono-, di-, and trimmer) of wild type, *fob1* and other mutants. Guarente's group have been argued that amount of accumulated ccc rDNA molecules in a mother cell determines her life span. (B) Mortality curves of wild type and *fob1* mutants. Life span was determined by scoring the number of daughter cells produced by each mother cell until she lost her ability to bud. Average life spans were as follows: wild type (black square), 17.9 generations and *fob1* (red circle), 28.2 generations.