Unequal Sister-Strand Recombination within Yeast Ribosomal DNA Does Not Require the RAD 52 Gene Product

Timothy J. Zamb and Thomas D. Petes

Department of Microbiology, The University of Chicago, 920 East 58th Street, Chicago, Illinois 60637, USA

Summary. We have found that the RAD52 gene product, which is required for gene conversion and recombination in the yeast Saccharomyces cerevisiae, is not required for unequal mitotic sister-strand recombination.

Key words: Intrachromosomal recombination — RAD52

Introduction

Recombination in eukaryotes is usually described as an exchange of genetic material between two homologous chromosomes. The exchange event involves non-sister chromatids (chromatids attached to separate centromeres). This type of recombination will be referred to as "interchromosomal" recombination. A second type of genetic exchange is recombination between sister chromatids (chromatids attached to the same centromere). Sister chromatid exchange, which is an intrachromosomal event, has been detected in Drosophila (Tartof 1973), in cultured mammalian cells (reviewed by Latt and Schreck 1980) and in yeast (Petes 1980; Szostak and Wu 1980). Although both interchromosomal and intrachromosomal recombination can occur within the same cell, it is not clear that the same mechanisms are used for both types of recombination. To investigate this problem, we have examined the effect of a mutation in the RAD52 locus on mitotic sister strand recombination. Previous workers (Game et al. 1980; Prakash et al. 1980; Malone and Esposito 1980) have suggested that the RAD52 gene product is required for both spontaneous and induced interchromosomal recombination and gene conversion. As described below, we have found that the RAD52 gene product is not required for mitotic sister-strand recombination.

The yeast genes in which we examined sister-strand recombination were the repeated genes coding for ribosomal RNA. In yeast, there are about 100 copies of these genes (Schweizer et al. 1969). These sequences are arranged in a single tandem array located on chromosome XII (Petes 1979).

In previous experiments (Petes 1980; Szostak and Wu 1980), methods were developed to examine sister-strand recombination within the ribosomal DNA (rDNA) gene cluster. The procedure involved inserting a selectable single-copy yeast gene into the tandem array by using recombinant plasmids and the yeast transformation technique (Hinnen et al. 1978). In these experiments, recombinant plasmids were constructed that contained the yeast LEU2 gene, yeast rDNA and bacterial plasmid sequences. When this plasmid was transformed into yeast strains mutant at the LEU2 locus, the LEU + transformants that were isolated contained the intact recombinant plasmid integrated in the rDNA gene cluster. This integration presumably occurs as the result of homologous recombination between the rDNA on the plasmid and rDNA in the yeast genome.

The stability of these insertions was analyzed. The presence or absence of the insertions was easily monitored by growth of the transformed cells in medium without leucine. It was found that the inserted LEU2 genes were frequently lost from the rDNA gene cluster in both meiosis (Petes 1980) and mitosis (Szostak and Wu 1980). For both meiotic and mitotic cells, it was shown that the mechanism of loss was unequal sister-strand recombination. Since the techniques used in these previous studies are similar to those reported here, we will discuss these experiments in detail.

An example of how unequal sister-strand recombination generates cells that are missing the LEU2 insertion is shown in Fig. 1. In this diagram, recombination in a haploid mitotic cell is drawn. Since the rDNA genes are...
Sister-Strand Exchange Does Not Require the RAD52 Gene

Leu² chromosome duplication

Unequal exchanges, each cell will grow into a clone of identical cells. Thus, if single yeast cells are plated onto rich growth medium, an unequal sister-strand recombination can give rise to a colony containing both LEU⁺ and leu⁻ cells. When such colonies are replica-plated to medium lacking leucine, sectored colonies are observed. Szostak and Wu (1980) showed, by Southern hybridization analysis, that the LEU⁺ side of such sectored colonies contained two copies of the LEU2 insertion per rDNA gene cluster. This is the result expected if the mitotic loss of LEU2 genes from the rDNA gene cluster occurred because of unequal sister-strand exchange.

In summary, the experiments described above showed that insertions within the rDNA gene cluster could be used to study unequal sister-strand recombination. Since the insertions are lost as a result of unequal sister-strand exchange and since this loss can be easily monitored by using the appropriate medium, we have used yeast strains carrying insertions in our analysis of the effect of RAD52 on sister-strand exchange.

Materials and Methods

Fig. 1. Unequal sister-strand recombination within the rDNA gene cluster of a haploid yeast cell. An insertion of the yeast LEU²⁺ gene is indicated by the small black rectangle. The small white rectangles represent single rDNA genes within the cluster. As the result of recombination between misaligned LEU²⁺ insertions, one rDNA gene cluster loses the insertion and the second receives a duplication of the insertion. Following recombination, these clusters will be segregated into different daughter cells. If the daughter cells divide clonally, a colony will be formed that has one sector of leu⁻ cells and one sector of LEU⁺ cells (with two LEU²⁺ insertions per cluster)

Strains. The yeast strain TP406 had the genotype: a leu2 ura3 his5 try1 gal2 form II rDNA (six EcoRI fragments per rDNA gene; Petes et al. 1978a). The strain RM43-9D had the genotype a rad52-1 ura3 form I (seven EcoRI fragments per rDNA gene; Petes et al. 1978a) and was provided by R. Malone (Loyola University). The transformed yeast strain TP406pss31(1) was obtained by treatment of TP406 with pss31 DNA (Hinnen et al. 1978). The diploid strain Z100 was constructed by mating TP406pss31(1) with RM43-9D.

The strain of E. coli that was used as a host for the plasmids MB1068 and pss31 was DB6656 (trp- lac- pyr F::urk-mk +) and was provided by C. Falco (M.I.T.).

Construction of the Plasmid pss31. The pss31 plasmid was constructed by S. Smolik-Utlaut (University of Chicago) from two other plasmids, MB1068 (provided by C. Falco, M.I.T.) and pY1rA12 (Petes et al. 1978a). The MB1068 plasmid has a HindIII fragment containing the yeast URA 3 + gene inserted into pBR322. The plasmid pY1rA12 has a complete form II yeast rDNA repeat inserted into the EcoRI site of pMB9 by "A's and Ts" tailing (Petes et al. 1978a). The pss31 plasmid was constructed by ligating a complete HindIII digest of MB1068 to a partial HindIII digest of pY1rA12. The ligation mixture was then used to transform the bacterial strain DB6656 by standard techniques (Petes et al. 1978b). Since the yeast URA 3 + gene complements the bacterial pyrF mutation (Bach et al. 1979), transformants are easily selected. A restriction map of pss31 is given in Fig. 2.

Fluctuation Analysis of the Loss of URA 3 + Gene. The procedure for the fluctuation analysis was similar to that described by Szostak and Wu (1980). Stationary phase populations of the URA 3 + spore segregants were diluted to a concentration of approximately 3 cells/ml and divided into 300 0.1 ml aliquots. The small cultures were incubated at 32°C for about 15 h to allow 6 to 10 cell divisions. Cultures were then plated on rich growth medium (YPD, Mortimer and Hawthorne 1969) and allowed to

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