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Genetics

# Recombination between sequences in nonhomologous positions

(chromosome rearrangements/translocations/gene conversion/ribosomal DNA)

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ABSTRACT **Crossing-over between the dispersed repeated sequences found in eukaryotic genomes would generate chromosomal rearrangements. The stability of the yeast genome suggests the existence of some constraint on the ability of these sequences to interact by recombination. We have constructed strains with two alleles of the *his3* gene located on different chromosomes. Gene conversion accounts for the majority of the recombination events between these genes, but about 10% of the events are crossovers that result in a reciprocal translocation. When one of the alleles is on an autonomously replicating centromere plasmid, recombination is 5- to 10-fold more frequent than when both alleles are chromosomal, suggesting that higher-order chromosome structure may play a role in restricting interchromosomal recombination. We have also used the translocation to deduce the orientation of the *his3* and rRNA genes relative to their centromeres.**

Eukaryotic cells contain moderately and highly repeated DNA sequences that are dispersed throughout the genome. The nature and frequency of the recombination events that take place between such dispersed sequences have important consequences for genomic stability. Gene conversion events have been postulated to account for the conservation of sequence homology between dispersed repeated elements (reviewed in refs. 1 and 2). In yeast, gene conversion is associated with a high frequency of crossing-over, in both meiotic (3) and mitotic (4) recombination. Crossovers between dispersed repetitive elements would generate chromosomal aberrations, which are deleterious either directly or in subsequent meioses. One might therefore expect to find severe constraints on the ability of dispersed repeats to interact by reciprocal recombination.

We have studied this problem in the yeast *Saccharomyces cerevisiae*, in which rare recombination events can easily be selected and characterized. Several classes of dispersed repeated sequences, ranging from two to hundreds of copies, have been identified in yeast (5–12). If these repeated elements were free to recombine with each other, translocations and other genomic rearrangements should be common in yeast. In fact, translocations in yeast are rare and may not arise from homologous recombination events. One translocation was found as a difference between two yeast strains of different background (13), and is of unknown nature. A second arose during selection for increased expression of the *cyc7* gene and does not appear to be due to homologous recombination (14). A third occurred as a revertant of a Ty*1* insertion mutation (15).

Gene conversion events have been reported between dispersed repeats on different chromosomes in yeast: between the *cyc1* and *cyc7* genes (16), between tRNA genes (17), and between copies of the *his3* gene placed on different chromosomes (18), but no reciprocal exchanges were seen. We examined the question of whether dispersed repeats can undergo crossing-over by selecting for recombination between dispersed copies of the *his3* gene. We find that these sequences can recombine with each other, but at a low rate, suggesting the existence of a constraint on the ability of sequences in nonhomologous positions to interact. Furthermore, this constraint appears to be relaxed when one of the two recombining sequences is on a centromere-containing plasmid.

A preliminary analysis of the translocation described here has been reported as part of a general method for constructing chromosomal rearrangements (19). Recombination, including crossing-over, between sequences on different chromosomes has recently been reported by two other laboratories (20, 21).

## MATERIALS AND METHODS

The double point mutations *leu2-3,112* and *his3-11,15* were derived from strain LL20 (*α*, *leu2-3,112*, *his3-11,15*, *can1*), and the markers *asp5*, *ura4*, and *can1* were from strain 6657-40; both were from the laboratory of G. Fink. *tcm1* was from strain CLP-1 (J. Davies), and *trp1* and *ade1* were from strain X4001-39A (M. Olson). All of the markers were crossed into the LL20 genetic background to give the two haploid strains D153-13C (*a*, *his3-11,15*, *leu2-3,112*, *asp5*, *ura4*, *can1*) and D154-13A (*α*, *his3-11,15*, *leu2-3,112*, *ade1*, *trp1*, *tcm1*).

Media, genetic methods, mutagenesis, and yeast transformation were as described (22). Trichodermin plates contained trichodermin (the gift of W. Gotfredson) at 20 μg/ml. Plasmid constructions and Southern blots were done as described (23).

The *his3-11,15* double point mutation was used because of its low reversion rate. To eliminate the possibility that one of the point mutations had reverted in the process of constructing strains D214 and D231, the presence of both mutations was confirmed. Spontaneous mitotic segregants that had lost the integrated plasmid by homologous recombination between flanking ribosomal DNA (rDNA) sequences were isolated and sporulated. Four spores from one tetrad were crossed to *his3-11* and *his3-15* tester strains, and the diploids were tested for the generation of histidine-independent ($His^+$) recombinants after UV irradiation. *his3-11/his3-15* control diploids gave rise to $His^+$ recombinants at a high frequency, whereas diploids derived from a cross to a strain carrying the double point mutation did not.

## RESULTS

To test the ability of homologous sequences on nonhomologous chromosomes to recombine, we constructed plasmids containing a defective allele of the yeast *his3* gene and integrated these plasmids at the yeast rDNA locus. Recombinants were selected between the *his3* gene in the rDNA locus and the *his3* gene at

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Abbreviations: rDNA, ribosomal DNA (DNA encoding rRNA); kb, kilobase(s); $His^+$, histidine-independent; $Leu^+$, leucine-independent.

the normal *his3* locus on chromosome XV. These recombinants were then analyzed genetically to determine the nature of the genomic rearrangements that occurred as a result of the recombination event.

**Insertion of *his3* at the rDNA Locus.** Plasmids containing a defective allele of the *his3* gene, a fragment of rDNA, and the *LEU2* gene were constructed as described in Fig. 1. We used a *his3* deletion constructed by K. Struhl (24) as a nonreverting *his3* allele. The deletion removes part of the coding region of the *his3* gene and replaces all sequences on the 5′ side with about 300 base pairs of λ phage DNA. As a result there is *his3* homology on only one side of the deletion. The 1.4-kb *Bam*HI fragment containing the *his3* allele was inserted in both orientations, yielding pSZ80 and pSZ81.

Strains with these plasmids integrated into the rDNA locus were constructed as follows. Strains D153-13C and D154-13A were made by standard crosses. The diploid obtained by crossing these strains is heterozygous for two centromere-linked markers (*ade1* and *trp1*) and for markers flanking the rDNA region (*asp5*, *rad5*, and *ura4*) but is homozygous for *leu2-3,112* and *his3-11,15*. This diploid sporulates well and has greater than 95% spore viability. D153-13C was transformed with pSZ80 and pSZ81, and six independent stable transformants from each plasmid were analyzed further. We wished to use a transformant with a single copy of the plasmid integrated at the rDNA locus. Total genomic DNA was prepared from each transformant, digested with the restriction enzyme *Xba* I, blotted by the method of Southern, and hybridized with a pBR322 probe. *Xba* I does not cut either pSZ80 or pSZ81 but does cut flanking rDNA sequences. The integration by homology of a single copy of either plasmid within the rDNA cluster results in a 15.8-kb *Xba* I fragment. Integration at other loci or multiple tandem integration at the rDNA locus results in *Xba* I fragments of different sizes. We identified transformants, from each plasmid, with a single copy integrated at the rDNA locus (data not shown). These transformants were shown to be haploids by crossing them with D154-13A. The resulting diploids showed 95% spore viability and 2:2 segregation of all markers, including the integrated plasmid. We chose one pSZ80 transformant and one pSZ81 transformant for further study. Each was crossed with D154-13A, and the resulting diploids (D231 and D214, respectively) were used for the selection and analysis of His$^+$ recombinants. The relevant aspects of the genotype of D214 are shown in Fig. 2. The only difference between D214 and D231 is in the orientation of the *his3* deletion fragment relative to the centromere of chromosome XII.

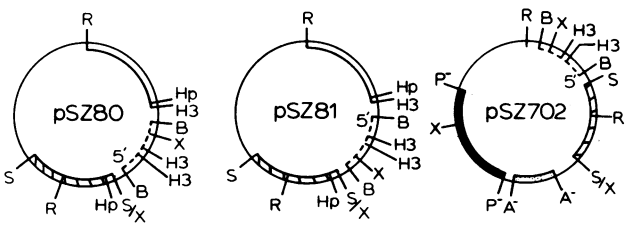


FIG. 1. Plasmids used in this study. pSZ80 and pSZ81 were constructed by inserting a rDNA fragment (double lines) containing the 5S RNA gene, a fragment containing a *his3* gene with a deletion of its 5′ end (from YRp7-Sc2712) (broken lines), and a *LEU2* fragment (hatched areas) from pYeLeu10 into pBR322 (single lines). The 5′ indicates the 5′ end of the *his3* gene. Both plasmids are 10.3 kilobases (kb). pSZ702 contains the same *his3* and *LEU2* segments inserted into YCp50, which is pBR322 containing *CEN4* (solid area) and *URA3* (stippled area). Restriction sites are R, *Eco*RI; B, *Bam*HI; H3, *Hin*dIII; Hp, *Hpa* I; B, *Bam*HI; P, *Pvu* II; X, *Xho* I; S, *Sal* I; and A, sites at which *URA3* was inserted by tailing with poly(dG).

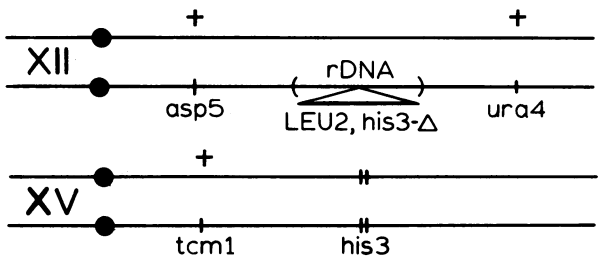


FIG. 2. Genotype of strains D214 and D231. Both strains are heterozygous for a *LEU2*, *his3*$^-$ deletion plasmid (pSZ81 or pSZ80) integrated into the rDNA locus on chromosome XII and homozygous for the double point mutation *his3-11,15* on chromosome XV.

**Selection and Analysis of His$^+$ Recombinants.** Approximately 70 independent His$^+$ recombinants were selected from D214 and D231. YPD plates (nonselective medium) were spread with about 200 cells per plate and incubated for 2–3 days at 30°C. The colonies were replica plated onto complete medium lacking histidine and incubated at 30°C; all of the colonies were His$^-$. After 3–6 days, His$^+$ papillae appeared on some of the colony prints. One papillus from each colony print was picked and purified on YPD agar. The purified His$^+$ recombinants were analyzed by tetrad dissection. Each strain was grown as a patch and replica plated onto sporulation medium; 5–10 tetrads were dissected from every strain that sporulated. Additional tetrad dissection and genetic analysis were done when necessary to define the event that gave rise to a His$^+$ recombinant. Several classes of recombination events were identified; these are described below and in Table 1.

For both diploids the largest class of events was due to gene conversion of the double point mutation *his3-11,15* to *HIS3*$^+$ (Table 1). Recombinants of this type were identified by their characteristic 2$^+$:2$^-$ segregation of both *leu2* and *his3*. The two genes segregated independently, with leucine independence (Leu$^+$) mapping to the rDNA locus on chromosome XII, and His$^+$ mapping to the *his3* locus on chromosome XV. The good spore viability in tetrads from these diploids and from the backcrosses used to map His$^+$ and Leu$^+$ indicated that no large chromosomal rearrangement had taken place. Additional genetic analysis has shown that these events are not due to double cross-overs. Such events would result in the transfer of the *his3* point mutations to the *his3* information on the plasmid in the rDNA locus. We showed that this had not occurred by crossing the integrated plasmid away from the *HIS*$^+$ gene and into a *his3-11,15* background. In all cases, such strains formed His$^+$ recombinants at the same frequency as a control strain constructed with the original integrated plasmid. The structure of several gene conversion recombinants was confirmed by Southern blot restriction mapping. No alterations in the structure of either *his3* gene had occurred (Fig. 3). Gene conversion of the *his3* deletion allele could not occur because of the lack of *his3*

Table 1. Number of His$^+$ recombinants per class

| Class | No. recombinants D214 | No. recombinants D231 |
|---|---|---|
| Gene conversion | 29 | 51 |
| Reciprocal translocation | 4 | 0 |
| Transposition | 1 | 1 |
| Homozygous for *leu2*$^-$, *LEU2*$^+$, or *HIS3*$^+$ | 9 | 11 |
| No sporulation | 10 | 11 |
| Others | 7 | 2 |
| Total | 60 | 76 |

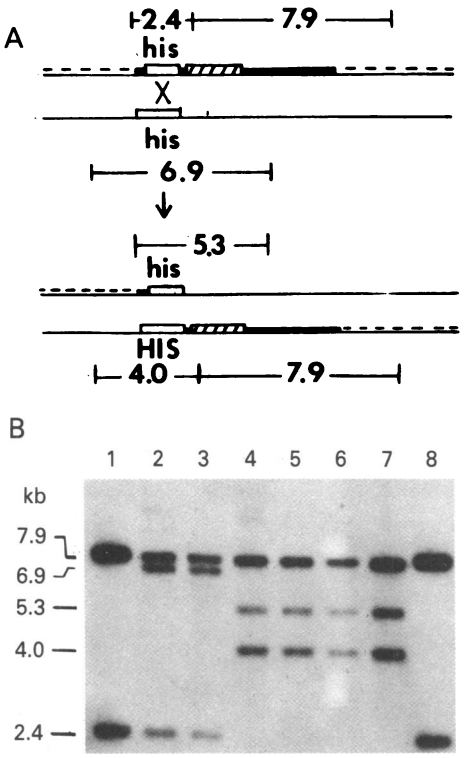


FIG. 3. Southern blot analysis of normal and translocation strains. (*A*) The parental strains D214 and D231 yield *Hpa* I fragments containing *his3* and rDNA of 2.4 and 7.9 kb from chromosome XII, and of 6.9 kb from chromosome XV. In translocation derivatives, rearranged fragments of 5.3 and 4.0 kb are predicted, as well as the constant 7.9-kb fragment. Broken line, rDNA; double line, *his3*; solid area, pBR322; hatched area, *LEU2*. (*B*) Southern blot analysis of *Hpa* I-cleaved genomic DNA with a *his3*, pBR322 probe. Lanes 1 and 8 are size markers of 2.4 and 7.9 kb from *Hpa* I-cleaved pSZ81. Lane 2, D214-9D, a spore from the parental strain D214; lanes 3–7, $His^+$ spores from strains described in the text; lane 3, gene convertant D214R13-4A; lane 4, plasmid transposition strain D214R6-5C; lane 5, reciprocal translocation D214R3S3-2C; lanes 6 and 7, translocation revertants D214R3S1-1D and D214R3S3-S104-22B.

homology on the 5′ side of the deletion. If such a conversion had occurred, $His^+$ and $Leu^+$ would have cosegregated and both would have mapped to the rDNA locus; no such events were observed.

The second class of recombinants we identified were reciprocal translocations resulting from a crossover between the two *his3* sequences. Four such recombinants were identified on the basis of their spore viability and marker segregation patterns. A reciprocal translocation, with both breakpoints not centromere linked, should yield tetrads with 4, 3, 2, 1, and 0 viable spores in a ratio of 1:0:4:0:1 (14). Dissection of the recombinant D214R3S3 resulted in 57:9:166:12:49 such tetrads, close to the expected ratio.

The *HIS3* and *LEU2* genes were tightly linked in the translocation strain D214R3S3. They segregated 2:2 in the four-spored tetrads, showing that each functional gene was present in a single location. The segregation was 1:1 in over 97% of the two-spored tetrads; this result maps the *HIS3* and *LEU2* genes to the translocation breakpoint. An analysis of the genetic properties of this reciprocal translocation is described below. The structure of the reciprocal translocation was confirmed by Southern blot restriction mapping, which showed the expected changes in the sequences surrounding the two *his3* genes (Fig. 3).

A third class of $His^+$ recombinants involves "transposition" of the integrated plasmid from the rDNA locus to the *his3* locus. Two such events were analyzed in detail. D231R76 showed 2:2 segregation for both *HIS3* and *LEU2*, with the two wild-type alleles being completely linked. Crosses to $HIS3^+$ strains showed that both $His^+$ and $Leu^+$ mapped to the *his3* locus and that no major chromosomal rearrangements were present in this strain. D214R6 showed $4^+:0^-$, $3^+:1^-$, and $2^+:2^-$ segregation for *LEU2*, and $2^+:2^-$ segregation for *HIS3*. All $HIS3^+$ spores were $LEU2^+$, suggesting that the strain contained two copies of the plasmid: one at the original location in rDNA and one at the *his3* locus. This was confirmed by a series of backcrosses in which the two copies of the plasmid were separated and mapped independently. This transposition event probably occurred in G2 of the cell cycle, followed by segregation of both plasmids (on chromosomes XII and XV) to the same daughter cell.

The remaining $HIS3^+$ recombinants fall into a number of diverse classes. Many recombinants had apparently experienced multiple events. For example, several $His^+$ recombinants, which probably arose as gene conversions between the two *his3* alleles, were homozygous for *HIS3* on chromosome XV, were homozygous *LEU2 ura4* or *leu2 URA4* on chromosome XII, or had lost the plasmid from chromosome XII. In one case, both markers flanking the rDNA locus (*ASP5* and *URA4*) became homozygous, while the plasmid integrated in the rDNA remained heterozygous. Four other $His^+$ recombinants harbored recessive lethal mutations that were unlinked to either the *his3* or rDNA loci. The origin and nature of these mutations is unknown. One recombinant was mitotically unstable for $His^+$; it and several other recombinants had unusual spore viability patterns that remain uninterpreted. Finally, many of the recombinants (15%) failed to sporulate, for unknown reasons.

**Properties of the Reciprocal Translocation.** If the translocation arose by a crossover between *his3* sequences, then the $HIS3^+$ spores from a tetrad should contain translocation chromosomes, while the $his3^-$ spores should contain wild-type chromosomes. This was tested by crossing spores of the translocation heterozygote D214R3S3 with wild-type tester strains. All diploids derived from the $His^-$ spores had good spore viability, whereas most of the diploids derived from the $HIS3^+$ spores had spore viability patterns characteristic of a translocation. Unexpectedly, several of these diploids had good spore viability. This appears to be due to reversion of the translocation as described below. When pairs of translocation spores were crossed, good spore viability was restored as expected of a translocation homozygote (Table 2, D420). The spores from one complete tetrad of this strain were tested by crosses to wild-type strains; all inherited the translocation.

To confirm the structure of the translocation, we showed that *tcm1* (chromosome XV) was mitotically linked to *ura4* (a chromosome XII marker normally unlinked to *tcm1*). The homozygous translocation, D420, with the genotype *tcm1*, *ura4*/*TCM1*, *URA4* was constructed. In this strain, selection for trichodermin-resistant recombinants (*tcm1*/*tcm1*) results in over

Table 2. Spore viabilities of tetrads from translocation strains and derivatives

| Description | Strain | 4:3:2:1:0 viable spores/tetrad |
|---|---|---|
| Original isolates | D214R3 | 10:2:9:3:2 |
| | D214R29 | 11:1:10:0:5 |
| | D214R39 | 12:4:11:0:5 |
| | D214R41 | 5:2:15:1:3 |
| Translocation subclone | D214R3S3 | 57:9:166:12:49 |
| Translocation revertants | D214R3S1 | 21:3:3:0:1 |
| | D214R3S3-S104 | 34:2:0:0:4 |
| Translocation homozygote | D420 | 74:4:1:0:1 |

70% of the *tcm1* homozygotes being homozygous for *ura4*, whereas in nontranslocation strains only 1% are *ura4/ura4*. Thus *tcm1* and *ura4* are on one chromosome in the translocation strain.

The initial characterization of the translocation was complicated by the presence of a subpopulation of cells that had good spore viability when sporulated. For example, D214R3 had an excess of tetrads with four viable spores. A subclone, designated D214R3S1, had a spore viability pattern of mainly 4 viable spores per tetrad. All markers segregated $2^+:2^-$. Other sucblones, such as D214R3S3, had spore viability patterns close to the theoretical ratio for a translocation heterozygote (Table 2). We observed that diploids heterozygous for *tcm1* and the translocation showed a frequency of homozygosis of *tcm1* 1/5th to 1/10th of that for diploids homozygous for either wild-type or translocation chromosomes. Of 108 mitotic subclones of the translocation heterozygote D214R3S3, one showed enhanced recombination to trichodermin resistance; it had good spore viability (Table 2, D214R3S3-S104). We believe that these strains are revertants of the translocation and arose by a crossover between the flanking rDNA (Fig. 4*B*). There are over 100 rDNA repeats; this extensive homology may explain the relatively high frequency of reversion. Revertants retain the translocation junctions as determined by Southern blot analysis (Fig. 3). Furthermore, when revertant strains were crossed with wild-type strains, good spore viability was seen and *URA4* mapped to chromosome XII.

The properties of the translocation revertant raised the possibility that the previously described plasmid transpositions were actually translocation revertants (Fig. 4). This difference cannot be distinguished genetically; however the observation of small rDNA circles in yeast (25) led us to expect the transposition of a small number of rDNA repeats to the *his3* locus if a circular intermediate was involved. We measured the number of rDNA repeats at the *his3* locus (Fig. 5) and found that only the rDNA present on pSZ81 had moved to the *his3* locus in transposition strain D214R6; strain D231R76 contained one additional rDNA repeat unit at the *his3* locus. In contrast, large stretches of rDNA have moved to the *his3* locus in the translocation revertants. Hence we believe that we are observing two distinct classes of events, plasmid transposition and translocation–reversion.

Additional genetic analysis of the reciprocal translocation has allowed us to deduce the orientations of the *his3* and rRNA genes relative to their respective centromeres. The *his3* deletion allele inserted into the rDNA locus on pSZ81 must be in the same orientation as the *his3* gene on chromosome XV, because a translocation and not a dicentric was recovered. The two possible orientations are illustrated in Fig. 6; the orientation determines whether *HIS3* and *LEU2* become attached to centromere XV or XII in the translocation derivative. Mitotic mapping on homozygous translocation diploids established that both *HIS3* and *LEU2* are attached to centromere XV in the translocation strain. From the known directions of transcription relative to their restriction maps (26, 27) the *his3* and 5S rRNA genes are oriented 5′ to 3′ away from their centromeres.

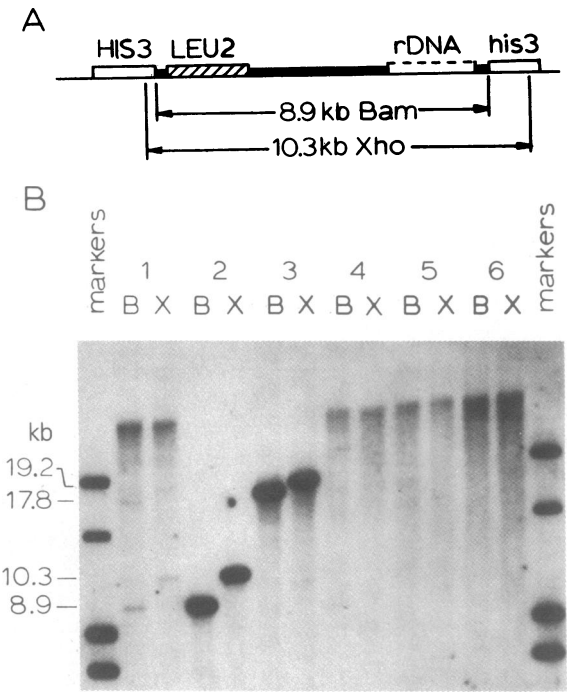


FIG. 5. (*A*) Size of restriction fragments generated after transposition of pSZ80 to the *his3* locus. Each additional rDNA repeat included in the rDNA segment increases the size of the fragments by 8.9 kb, because neither enzyme cuts within rDNA. (*B*) Southern blot hybridization, with a pBR322 probe, of genomic DNA cut with either *Bam*HI (B) or *Xho* I (X). Lanes 1, D214-9D, a spore from the parental strain D214. The faint bands are due to unequal crossing-over within the rDNA. Most of the hybridization is at high molecular weight, because the rDNA is not cut. Lanes 2 and 3, plasmid transposition strains D214R6-5C and D231R76-18D, showing movement of 0 and 1 rDNA repeats. Lanes 4, 5, and 6, translocation revertants D214R3S1-1D, D214R3S3-S104-22B, and D417-1D. So much rDNA has moved that only high molecular weight DNA hybridizes to the probe.

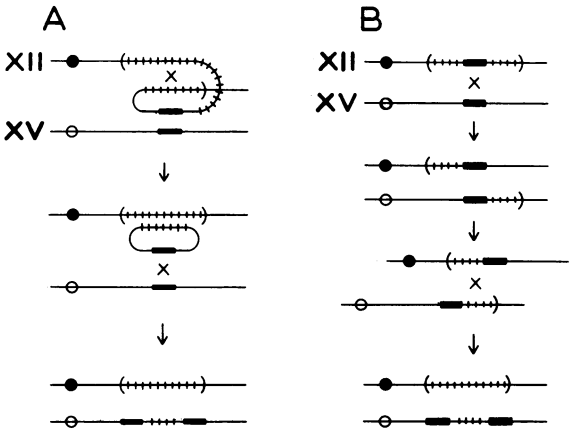


FIG. 4. Alternative pathways for the transposition of a plasmid integrated at the rDNA locus to the *his3* locus. (*A*) The plasmid excises as a circle, which reintegrates at *his3*. (*B*) A translocation is generated, but it reverts to the wild-type configuration by recombination.

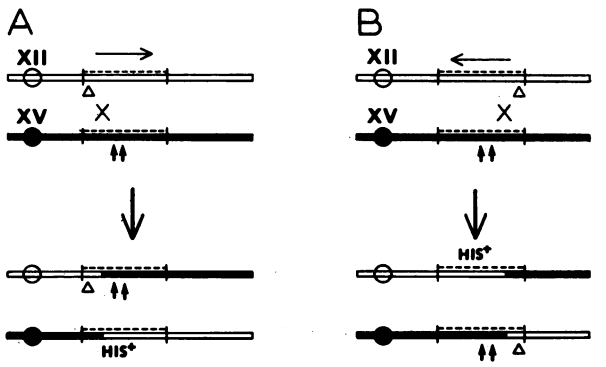


FIG. 6. Orientations of the *his3* and rRNA genes. The *his3* genes on chromosomes XII and XV in strain D214 are in the same orientation. If they are oriented as in *A*, the *HIS*$^+$ gene produced by crossing-over is linked to centromere XV. If the orientation is as in *B*, the *HIS*$^+$ gene is linked to centromere XII. Broken line, *his3*; horizontal arrows, direction of transcription; pairs of vertical arrows, double point mutation; Δ, 5′ deletion.

**Plasmid–Chromosome Recombination.** Recombination between an autonomously replicating centromere plasmid and a chromosome was compared with recombination between two nonhomologous chromosomes. We inserted the *his3* deletion fragment and the *LEU2* fragment used in the experiments described above into the centromere-containing plasmid YCp50 (from C. Mann). The resulting plasmid, pSZ702 (Fig. 1), is maintained as a stable, autonomous plasmid in yeast. It is maintained at a copy number of close to one as measured by Southern blot hybridization and tetrad dissection. Strain D719 containing this plasmid was constructed by transforming D153-13C with pSZ702 and crossing it with D154-13A. D719 is isogeneic with D214 and D231, except that the *his3* deletion allele is on a centromere plasmid instead of integrated at the rDNA locus.

The rates with which D719, D214, and D231 gave rise to $His^+$ recombinants were determined by fluctuation analysis, using the method of the median of Lea and Coulson (28). The rates for D214 and D231 were quite similar ($2.4 \pm 0.5 \times 10^{-8}$ and $1.3 \pm 0.3 \times 10^{-8}$ per cell division, respectively), but the rate for the plasmid-containing strain was 5- to 10-fold higher ($10.7 \pm 1.5 \times 10^{-8}$ per cell division).

## DISCUSSION

We have analyzed recombination events between two copies of the *his3* gene located on nonhomologous chromosomes. The major class of events was due to gene conversion, but about 10% of the events involved one or more reciprocal exchanges (crossovers), which were observed as reciprocal translocations or transpositions of an integrated plasmid. This level of crossing-over relative to gene conversion is similar to that observed for intrachromosomal mitotic recombination between direct repeats of the *his4* gene (29). Interchromosomal gene conversion has been postulated to be involved in the maintenance of sequence homogeneity between dispersed repeats in yeast and higher eukaryotes. If the association between conversion and crossing over is general, the influence of deleterious chromosomal rearrangements that accompany conversion must be taken into account in models of concerted evolution.

Genetic analysis of our translocation allowed us to determine the orientation of the *his3* and rRNA genes; our results agree with those of Zamb and Petes (30). Recently, two laboratories have reported (20, 21) the generation of translocations by procedures similar to those described here. Potier *et al.* (21) isolated a *ura2*:*his3* translocation. From their data and ours, we can conclude that *ura2* is transcribed away from its centromere.

We observed two recombinants that appear to have resulted from the excision of a plasmid integrated at the rDNA locus and its reintegration at the *his3* locus. These events are probably not translocation reversions because they resulted in the transfer of only 0 and 1 rDNA repeat to the *his3* locus, whereas the translocation reversions resulted in the transfer of a large number of rDNA repeats. This observation is consistent with physical observations of small rDNA circles (25) and suggests a role for such circles in shuffling repeat units within the rDNA cluster.

Many of the recombinants that we analyzed appear to have resulted from multiple events involving one region of DNA. A possible explanation for this observation is that lesions in the DNA remaining after the completion of one event stimulate secondary events.

The low frequency of interchromosomal recombination that we observed relative to plasmid–chromosome recombination suggests that recombination between nonhomologous chromosomes is limited by some aspect of higher-order chromosome structure. If, for example, long homologous stretches are required for pairing to occur, two *his3* genes on different chromosomes may only rarely become paired and recombine. Alternatively, some aspect of chromosomal structure could impede pairing between sequences on different chromosomes. If each chromosome is coiled in one domain, interactions between chromosomes could be minimized. More simply, the large size of a chromosome could decrease diffusion to the extent that contacts between homologous sequences on different chromosomes are minimized. However, we cannot eliminate the possibility that the free plasmid shows high recombination levels for some unrelated reason, such as the presence of *CEN4* or *URA3* sequences, some aspect of plasmid structure, or an inhibitory influence of the rDNA cluster on recombination. The system that we have described for studying recombination between nonhomologous chromosomes may provide an opportunity for obtaining mutants defective in higher-order chromosome structure; these might have the phenotype of being hyper-recombinant specifically for interchromosomal recombination.

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