CHROMOSOMAL TRANSLOCATIONS GENERATED BY HIGH-FREQUENCY MEIOTIC RECOMBINATION BETWEEN REPEATED YEAST GENES

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Manuscript received May 29, 1986
Accepted July 25, 1986

ABSTRACT

We have examined meiotic and mitotic recombination between repeated genes on nonhomologous chromosomes in the yeast *Saccharomyces cerevisiae*. The results of these experiments can be summarized in three statements. First, gene conversion events between repeats on nonhomologous chromosomes occur frequently in meiosis. The frequency of such conversion events is only 17-fold less than the analogous frequency of conversion between genes at allelic positions on homologous chromosomes. Second, meiotic and mitotic conversion events between repeated genes on nonhomologous chromosomes are associated with reciprocal recombination to the same extent as conversion between allelic sequences. The reciprocal exchanges between the repeated genes result in chromosomal translocations. Finally, recombination between repeated genes on nonhomologous chromosomes occurs much more frequently in meiosis than in mitosis.

RECOMBINATION in eukaryotic organisms occurs in mitosis and meiosis and may be either a reciprocal or nonreciprocal event. Reciprocal recombination changes the linkage relationships of genes along a chromosome and is the basis of genetic mapping. In those organisms in which all four products of a single meiotic division can be analyzed, nonreciprocal recombination or gene conversion is signaled by a 3:1 segregation pattern of allelic sequences instead of the normal 2:2 segregation pattern. In fungi, reciprocal and nonreciprocal recombination are associated with one another so that, on the average, a gene conversion event at a given locus is accompanied by a reciprocal exchange event approximately half of the time (reviewed by ORR-WEAVER and SZOSTAK 1985). These conversion-associated reciprocal exchanges are usually detected by examining genetic markers that flank the site of the conversion event. The association of reciprocal and nonreciprocal recombination has been interpreted to mean that the two processes are mechanistically related to one another, and this notion has been incorporated into all current models of genetic recombination (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Although models differ in the method of initiating a recombination event, all

models propose that the mode of resolution of a structural intermediate manifested as a gene conversion event determines whether the conversion event is accompanied by a reciprocal exchange.

Recombination is usually thought of as occurring between genes at identical positions on homologous chromosomes (defined here as allelic recombination), but it may also occur between repeated genes dispersed throughout the genome. Gene conversion events between dispersed repeats are thought to be important in the concerted evolution of multigene families (EDELMAN and GALLY 1970) and have been implicated in the generation of the high degree of polymorphism characteristic of the major histocompatibility complex (BALTIMORE 1981). Reciprocal exchanges between dispersed repeats will result in chromosome rearrangements (inversions, deletions and translocations) that are likely to be evolutionarily important. Recombination between naturally occurring and artificially constructed dispersed repeats is well documented in the yeast *Saccharomyces cerevisiae*. There are numerous examples of recombination events involving dispersed repeats on the same chromosome in both meiosis (KLEIN and PETES 1981; ROEDER 1983; KLEIN 1984; JACKSON and FINK 1985) and mitosis (JACKSON and FINK 1981; KLAR and STRATHERN 1984; ROEDER, SMITH and LAMBIE 1984). Several groups have described recombination events between repeats on nonhomologous chromosomes during mitotic growth (SCHERER and DAVIS 1980; ERNST, STEWART and SHERMAN 1981; MIKUS and PETES 1982; POTIER, WINSOR and LACROUTE 1982; SUGAWARA and SZOSTAK 1983; ROEDER, SMITH and LAMBIE 1984). We recently reported that gene conversion between repeated genes on nonhomologous chromosomes also occurs in meiosis (JINKS-ROBERTSON and PETES 1985).

In our previous work (JINKS-ROBERTSON and PETES 1985), we observed meiotic recombination between *HIS3* repeats on nonhomologous chromosomes at a frequency (approximately 0.5% in dissected tetrads) similar to that observed for allelic recombination events. Of the three events detected and analyzed in detail, all were gene conversions, and none of these conversion events was associated with reciprocal exchange of flanking markers. To examine this type of recombination in more detail, we have set up a system that allows us to detect such events genetically among a population of random spores. As described below, we have found that gene conversion events between repeated genes on nonhomologous chromosomes are often associated with reciprocal recombination of flanking DNA, thus resulting in chromosomal translocations. The meiotic frequency of these events is high enough that they are likely to be the major source of spontaneous chromosome rearrangements in yeast. We also demonstrate that, although similar recombination events occur in mitotically dividing cells, the frequency of events per mitotic cell division is about 1000-fold less than in meiosis.

MATERIALS AND METHODS

Media and growth conditions: Yeast strains were grown vegetatively at 32° and sporulated at room temperature. Standard media and genetic techniques were used (SHERMAN, FINK and HICKS 1982). YPD media (1% yeast extract, 2% Bacto-peptone, 2% dextrose, 3% agar) was used for nonselective growth. Nutritional markers were

TABLE 1

Plasmids and strains

Plasmid strain	Description
pSR7	1.7-kb *HIS3*$^+$-*Bam*HI fragment cloned into *Bam*HI site of pBR322
pSR10	5.5-kb *ura3-3 Bam*HI fragment inserted into *HIS3*$^+$ gene of pSR7
pSR12	*Bgl*II site upstream of *ura3-3* on pRB30 deleted
pSR13	5.5-kb *Bam*HI *URA3*$^+$ fragment cloned into *Bam*HI site of pBR322
pSR14	1.5-kb *Bam*HI *CEN5*$^+$ fragment cloned into *Bam*HI site of pBR322
pSR15	2.2-kb *Sal*I/*Xho*I *LEU2*$^+$ fragment cloned into *Sal*I site of M13mp7
pSR16	2.2-kb *Bam*HI *LEU2*$^+$ fragment from pSR15 inserted into *Bgl*II site of pSR14
DBY931	**a** *his4 leu2-3,112 ura3-50 met8-1 can1-101* (FALCO, ROSE and BOTSTEIN 1983)
SK124	*α asp5 lys7 his2 his3 trp1 ade2* (from R. EASTON ESPOSITO)
SJR36	**a** *his3 his4 leu2-3,112 ura3-50 met8-1 trp1 ade2*
SJR37	Spontaneous Met$^+$ derivative of SJR36; contains amber suppressor
SJR39	Spontaneous Can^r derivative of SJR36
SJR42	*his3* in SJR37 replaced with *his3::ura3-3* by transformation
SJR43	*ura3-50* in SJR37 replaced with *ura3-3ΔBgl* by transformation
SJR45	*MAT***a** derivative of SJR39
SJR52	Spore from SJR42 × SJR45 *α his3::ura3-3 his4 ura3-50 leu2-3,112 trp1 ade2 met8-1 CAN1*^{s}
SJR53	Spore from SJR43 × SJR45 *α his3 his4 ura3-3ΔBgl leu2-3,112 trp1 ade2 met8-1 CAN1*^{s}
SJR56	*CEN5*$^+$ of DBY931 replaced with *CEN5*$^+$-*LEU2*$^+$ by transformation
SJR58	SJR53 × SJR56 *α/***a** *his3/HIS3*$^+$ *his4/his4 ura3-3ΔBgl/ura3-50 leu2-3,112/leu2-3,112 trp1/TRP1*$^+$ *ade2/ADE2*$^+$ *met8-1/met8-1 CAN1*^{s}*/can1-101 CEN5*$^+$*/CEN5*$^+$-*LEU2*$^+$
SJR59	SJR52 × SJR56 *α/***a** *his3::ura3-3/HIS3*$^+$ *his4/his4 ura3-50/ura3-50 leu2-3,112/leu2-3,112 trp1/TRP1*$^+$ *ade2/ADE2*$^+$ *met8-1/met8-1 CAN1*^{s}*/can1-101*
SJR64	V:XV, XV:V translocation homozygote *α/***a** *leu2-3,112/leu2-3,112 CEN5/CEN5-LEU2*$^+$ *ADE2*$^+$*/ade2 TRP1*$^+$*/TRP1*$^+$ *met8-1/met8-1 CAN*^{s}*/can1-101 his3::ura3-3*$^{(+)}$*/his3::ura3-3*$^{(+)}$ *his4/his4 ura3-50*$^{(+)}$*/ura3-50*$^{(+)}$ Two of the four *ura3* genes are *URA*$^+$

scored on SD complete media missing one amino acid. For analyses of random spores, the SD plates were supplemented with five times the normal amount of leucine to prevent inadvertant selection against Leu$^-$ haploids. Canavanine resistance was scored on SD minus arginine plates supplemented with 50 μg/ml canavanine. For sporulation, diploid cells were grown vegetatively in YPA (1% yeast extract, 2% Bacto-peptone, 1% potassium acetate) and sporulated in SM (2% potassium acetate) supplemented with required amino acids.

E. coli strains were grown at 37° in LB (1% yeast extract, 0.5% Bacto-tryptone, 1% NaCl plus 1.5% agar for plates). The medium was supplemented with 50 μg/ml Ampicillin for growth of plasmid-containing strains.

Plasmid constructions: A complete list of plasmids constructed during this work is given in Table 1. *E. coli* strain HB101 was used as a host in all cloning experiments, and standard cloning procedures were used (MANIATIS, FRITSCH and SAMBROOK 1982). The plasmid pSR7 was constructed by inserting a 1.7-kb *Bam*HI fragment encoding the yeast *HIS3*$^+$ gene (from YEp6; BOTSTEIN *et al.* 1979) into the *Bam*HI site of pBR322.

The plasmid pSR10 contains an insertion of a 5.5-kb *Bam*HI fragment encoding the *ura3-3* gene (from pRB30; FALCO, ROSE and BOTSTEIN 1983) into one of the *Bgl*II sites within the *HIS3*$^+$ gene on pSR7. The *his3* allele thus constructed is called *his3*::*ura3-3*. The insertion of the *ura3-3* fragment was accomplished by ligating purified linear derivatives of pSR7 (generated by partial digestion with *Bgl*II) to *Bam*HI-digested pRB30. In pSR10, the *ura3-3* fragment is inserted at the promoter-proximal *Bgl*II site within the *HIS3*$^+$ gene (see STRUHL 1985); transcription of *URA3* and *HIS3* converge. The plasmid pSR12 is identical to the plasmid pRB30 (5.5-kb *Bam*HI *ura3-3* fragment inserted into the *Bam*HI site of YEp21) except for deletion of the single *Bgl*II site located approximately 2 kb upstream of the *ura3-3* gene. The plasmid pSR12 was constructed by digesting pRB30 with *Bgl*II, filling in the *Bgl*II-generated cohesive ends with the Klenow fragment of DNA polymerase I, and re-ligating the filled-in, blunt ends. The *ura3* allele thus constructed is called *ura3-3ΔBgl*.

The plasmid pSR16 contains an insertion of the yeast *LEU2*$^+$ gene into a 1.5-kb fragment containing the centromere of chromosome *V* (*CEN5*). This plasmid was constructed in several steps. First, a 1.5-kb *Bam*HI fragment containing *CEN5* function [from pYE(MMS1)3; MAINE, SUROSKY and TYE 1984] was cloned into the *Bam*HI site of pBR322 to give plasmid pSR14. Next, the plasmid pSR15 was constructed by inserting a 2.2-kb *Sal*I/*Xho*I fragment encoding the *LEU2*$^+$ gene (from CV9; PETES 1980) into the *Sal*I site of M13mp7. The 2.2-kb *LEU2*$^+$ fragment was then removed from pSR15 by digestion with *Bam*HI, and this fragment was subsequently inserted into the unique *Bgl*II site within the 1.5-kb *CEN5*-containing fragment of pSR14 to yield plasmid pSR16. The insertion thus constructed is called *CEN5*$^+$-*LEU2*$^+$. It should be noted that the insertion of the *LEU2*$^+$ gene has no apparent effect on *CEN5* function.

The plasmid pSR13 was constructed for use as a probe for *URA3*-homologous sequences in Southern blot analyses of yeast DNAs. This plasmid was constructed by inserting a 5.5-kb *Bam*HI fragment encoding the *URA3*$^+$ gene (from pRB90; obtained from S. C. FALCO) into the *Bam*HI site of pBR322.

Strain constructions: A complete list of strains used in this study is given in Table 1. Strain SJR36 (**a** *his3 his4 leu2-3,112 ura3-50 met8-1 trp1 ade2*) was isolated as a spore from a diploid strain constructed by mating strains DBY931 (**a** *his4 leu2-3,112 ura3-50 met8-1 can1-101*) and SK124 (*α asp5 lys7 his2 his3 trp1 ade2*). The *met8-1* allele is an amber mutation. In order to selectively transform SJR36 with the amber allele *ura3-3*, we isolated a derivative of SJR36 containing an amber suppressor by selecting for spontaneous Met$^+$ colonies. The Met$^+$ isolate used in subsequent constructions is called SJR37. Replacement of the *ura3-50* allele in strain SJR37 by *ura3-3ΔBgl* or the *his3* allele by *his3*::*ura3-3* was accomplished using the omega transformation procedure of ROTHSTEIN (1983). SJR37 was transformed to Ura$^+$ using *Bam*HI-digested pSR10 and *Bam*HI-digested pSR12 to yield the isogenic strains SJR42 (SJR37 *his3*::*ura3-3*) and SJR43 (SJR37 *ura3-3ΔBgl*), respectively. To get rid of the suppressor mutation in strains SJR42 and SJR43, we mated each strain to an isogenic strain of opposite mating type lacking the suppressor (SJR45). The mating-type switch was effected by transforming a spontaneous Can^r derivative of SJR36 (SJR39) with a plasmid containing the yeast HO gene (YEp-HO; from I. HERSKOWITZ). The isogenic haploid strains SJR52 (*α his3*::*ura3-3 his4 ura3-50 leu2-3,112 trp1 ade2 met8-1 CAN1*$^+$) and SJR53 (*α his3 his4 ura3-3ΔBgl leu2-3,112 trp1 ade2 met8-1 CAN1*$^+$), each of which contains the *ura3-3* gene but lacks an amber suppressor, were thus constructed.

The strain SJR56 (**a** *his4 leu2-3,112 ura3-50 met8-1 can1-101 CEN5*$^+$-*LEU2*$^+$) was constructed by transforming strain DBY931 with *Bam*HI-digested pSR16 and selecting Leu$^+$ transformants. The strains SJR52 and SJR53 were mated with SJR56 to give the diploid strains SJR59 and SJR58, respectively.

Meiotic experiments: Single colonies were inoculated into 10 ml of YPA and were grown to approximately 2 × 10^7 cells/ml. Cells were washed once with 10 ml of H_2O and were resuspended in 2 ml of H_2O. The suspensions were sonicated 5 sec to disperse clumps of cells, and appropriate dilutions were plated selectively on SD-uracil or non-

selectively on SD complete plates to determine the frequency of mitotically derived Ura$^+$ colonies. Colony counts were made after 3 days. The remainder of the cells were diluted into SM and sporulated. After 4 days in SM, random spores were prepared as described by DAVIDOW and BYERS (1984). Briefly, the sporulated cultures were treated with a reducing agent, followed by treatment with glusolase to digest the ascal wall and kill vegetative cells. The cultures were then sonicated to disperse the spores, and appropriate dilutions of the random spores were plated selectively on SD-uracil or nonselectively on SD complete plates. Colonies were counted after 5 days. Meiotically derived Ura$^+$ colonies were purified nonselectively on YPD before further genetic or physical analyses.

Mitotic experiments: For frequency determinations, single colonies were inoculated into YPA and were grown to approximately 2×10^7 cells/ml. Cells were washed once with 10 ml H_2O and sonicated for 5 sec before plating appropriate dilutions selectively on SD-uracil or nonselectively on SD complete plates. Colonies were counted after 3 days.

Independent Ura$^+$ colonies for analysis of conversion-associated reciprocal exchange were isolated as follows. Independent colonies were grown in patches on YPD plates and subsequently were replica-plated onto SD-uracil medium. Small Ura$^+$ colonies appeared on the selective plates after 5–7 days. A single Ura$^+$ colony was picked from each patch and purified nonselectively on YPD. A single purified colony was used for subsequent genetic and physical analyses.

Physical analysis of yeast DNAs: DNAs were prepared from 5 ml of stationary cells grown in YPD (SHERMAN, FINK and HICKS 1982). DNAs were digested with appropriate restriction enzymes, and fragments were separated by agarose gel electrophoresis. Fragments were transferred from the gels to nitrocellulose filters by the method of SOUTHERN (1975). The filters were probed for *URA3*-homologous sequences in Denhardt's solution at 58° for 16 hr. ^{32}P-labeled pSR13 DNA prepared by nicktranslation was used in all experiments as the probe. Radioactively labeled fragments were visualized by autoradiography.

RESULTS

Construction of yeast strains (SJR58 and SJR59) to monitor recombination between repeated genes on nonhomologous chromosomes: The experimental strain SJR59 was constructed in order to determine the relationship between meiotic gene conversion and reciprocal recombination when the interacting genes are on nonhomologous chromosomes. Two different mutant *ura3* genes (*ura3-3* on chromosome *XV* and *ura3-50* on chromosome *V*) were used as the interacting repeated sequences, and recombination between the repeats was detected by selecting for Ura$^+$ spores (uracil prototrophs) from a population of random spores. We assumed that a recombination event resulting in a *URA3*$^+$ gene was likely to be a nonreciprocal gene conversion event because previous studies have shown that the majority of intragenic recombination in yeast is nonreciprocal (FOGEL, MORTIMER and LUSNAK 1981). The strain SJR58 is isogenic to strain SJR59 and was constructed as a control for the experiments with SJR59. SJR58 has the same two mutant *ura3* genes as SJR59, but the genes are at allelic positions on chromosome *V* homologues, rather than on nonhomologous chromosomes. This pair of isogenic control and experimental strains allowed direct comparison of meiotic conversion events involving *ura3*$^-$ genes on homologous chromosomes and nonhomologous chromosomes with respect to absolute frequency and with respect to conversion-associated recombination of flanking markers. Analogous experiments were also done in mitot-

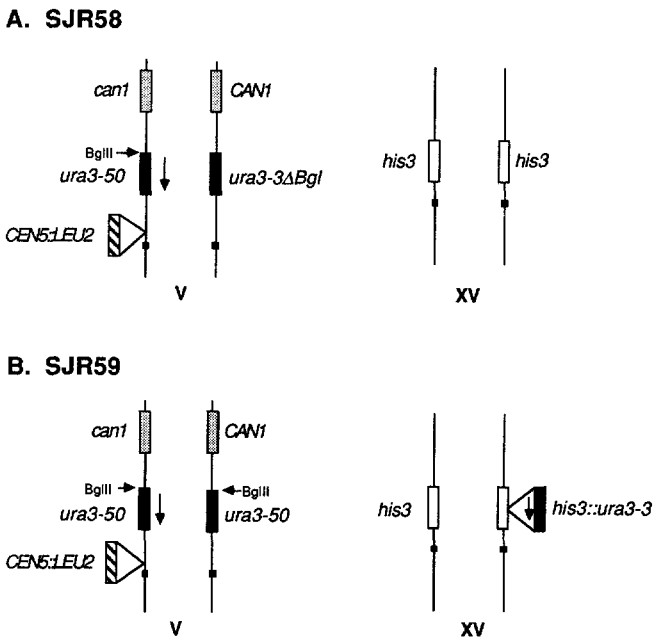

FIGURE 1.—Relevant genetic features of strains SJR58 and SJR59. The relevant genetic features of these strains are schematically illustrated in panels A and B, respectively. For simplicity, only chromosomes *V* and *XV* are shown. The important loci on these chromosomes are represented by rectangles and are named according to the allele present; small squares represent the centromeres. In the control strain SJR58, both mutant *ura3* genes are at the *URA3* locus on chromosome *V*; in the experimental strain SJR59, the *ura3-50* gene is on chromosome *V* and the *ura3-3* gene is inserted into the *HIS3* locus on chromosome *XV*. As the arrows indicate, all *URA3* genes are transcribed toward their respective centromeres. The *Bgl*II restriction site polymorphism, the centromere-linked *LEU2* polymorphism and the heterozygosity at the *CAN1* locus used to monitor conversion-associated reciprocal recombination of flanking markers on chromosome *V* in strain SJR58 are indicated.

ically dividing cells, thus allowing comparison of meiotic and mitotic events within each of the two strains.

The relevant features of strains SJR58 and SJR59 are schematically shown in Figure 1 (for details of the constructions, see MATERIALS AND METHODS). The control strain SJR58 has the *ura3-3* and *ura3-50* genes at the *URA3* locus on chromosome *V*. A *LEU2*$^+$ gene was inserted near the centromere of one of the chromosome *V* homologues, and a centromere-distal *Bgl*II restriction site was deleted on the other homologue. These two polymorphisms were used as flanking markers to monitor conversion-associated reciprocal exchange in Ura$^+$ random spores. Nonrecombinant spores maintain the parental configuration of flanking markers and are, therefore, either Leu$^+$ and have the *Bgl*II site (Leu$^+$Bgl$^+$) or Leu$^-$ and missing the *Bgl*II site (Leu$^-$ΔBgl). Ura$^+$ spores recombinant for the flanking markers are either Leu$^+$ΔBgl or Leu$^-$Bgl$^+$. An autoradiogram of a representative Southern blot of *Bgl*II-digested DNAs from Ura$^+$ random spores is shown in Figure 2 to illustrate this type of analysis. In addition to the *Bgl*II polymorphism, heterozygosity at the centromere-distal

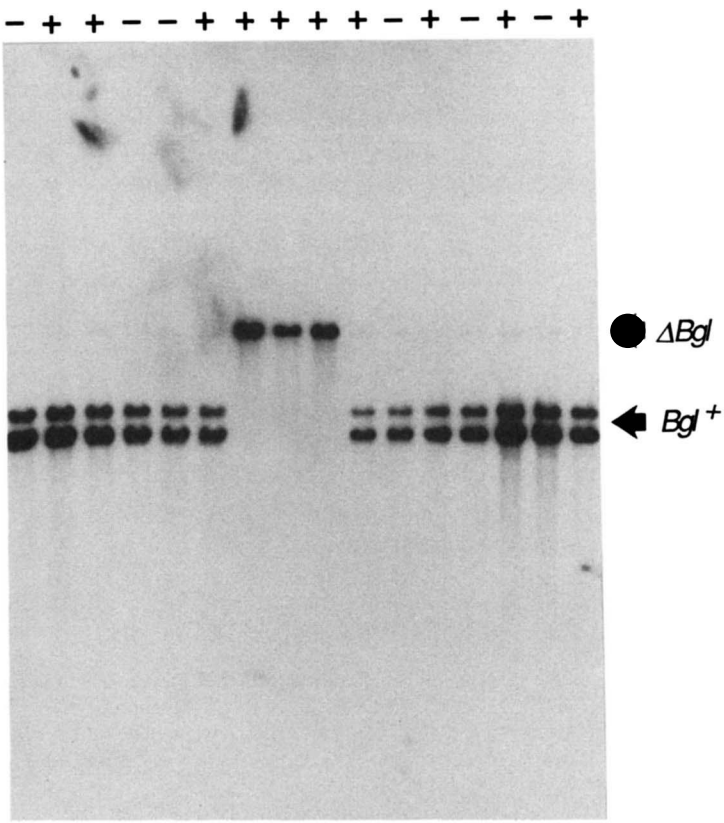

FIGURE 2.—Southern blot analysis of DNAs isolated from SJR58 Ura$^+$ random spores. Spore DNAs were digested with *Bgl*II and analyzed on a 0.5% agarose gel. Fragments were transferred to nitrocellulose, and *URA3*-homologous sequences were detected by hybridizing the blot with a 5.5-kb *Bam*HI fragment containing the *URA3* locus (plasmid pSR13). This *Bam*HI fragment contains the *Bgl*II site upstream of the *URA3* coding sequence that was deleted to produce the *ura3-3ΔBgl* allele. Thus, if the *Bgl*II site is present, two small fragments of approximately 4 kb hybridize to the probe; if the *Bgl*II site is missing, one large fragment of approximately 8 kb is detected. The Leu$^+$ or Leu$^-$ phenotype of each spore is indicated above the corresponding lane by a + or −, respectively.

CAN1 locus was also used in conjunction with the *LEU2* polymorphism to monitor conversion-associated recombination of flanking markers. Strains containing the wild-type *CAN1*^{s} allele are sensitive to the arginine analogue canavanine; strains containing the mutant allele *can1*^{r} are resistant to canavanine. Nonrecombinant spores are Leu$^+$Can^r or Leu$^-$Can^s; recombinant spores are Leu$^+$Can^s or Leu$^-$Can^r.

The experimental strain SJR59 has the *ura3-50* gene at the *URA3* locus on chromosome *V* and the *ura3-3* gene inserted at the *HIS3* locus (*his3::ura3-3*) on chromosome *XV*. In order to recover reciprocal exchanges between the mutant *ura3* genes as viable products, the repeats were oriented in the same direction relative to their centromeres. Recombination between two genes in the same orientation with respect to their centromeres yields a reciprocal trans-

location between the involved chromosomes; recombination between two genes in opposite orientations with respect to their centromeres is likely to be lethal because it yields a dicentric chromosome and an acentric fragment. Restriction enzyme sites that asymmetrically flank the *ura3*$^-$ repeats were used to monitor conversion-associated reciprocal exchange in Ura$^+$ spores. As illustrated schematically in Figure 3, the *ura3-50* gene at the *URA3* locus and the *ura3-3* gene at the *HIS3* locus are on *Eco*RI fragments of approximately 13 and 16 kb, respectively. Recombination between the *ura3*$^-$ repeats yields two new *Eco*RI fragments of approximately 9 and 20 kb. Figure 3 also shows a representative Southern blot of *Eco*RI-digested DNAs from SJR59-derived Ura$^+$ random spores that illustrates detection of the nonrecombinant and recombinant *Eco*RI fragments.

A final point that deserves mention concerns the two mutant *ura3* alleles present in strains SJR58 and SJR59. The *ura3-3* and *ura3-50* mutations are at opposite ends of the *URA3* coding sequence, with the *ura3-3* mutation being promoter-proximal (see FALCO, ROSE and BOTSTEIN 1983). The *ura3-50* mutation is nonrevertable and is thought to be the result of a small deletion; the *ura3-3* mutation is an amber mutation that reverts at a frequency of less than 5×10^{-7} (FALCO, ROSE and BOTSTEIN 1983). Two types of reversion of the *ura3-3* amber mutation are possible: true reversion to a wild-type *URA3*$^+$ gene and pseudoreversion due to the presence of an amber suppressor. Pseudoreversion accounts for more than 95% of the Ura$^+$ revertants isolated from *ura3-3* strains (FALCO, ROSE and BOTSTEIN 1983). To eliminate the problem of amber suppressor mutations in the analyses with strains SJR58 and SJR59, we constructed both strains to be homozygous for an additional amber mutation (*met8-1*). In all the experiments described below, Ura$^+$ colonies were screened for a Met$^+$ phenotype. Ura$^+$ Met$^+$ colonies were assumed to be pseudorevertants harboring an amber suppressor and were not included in the recombination data. It should be noted that, even in the mitotic experiments where the frequency of Ura$^+$ colonies was low, only a small fraction (<10%) of the Ura$^+$ colonies were also Met$^+$. Given that pseudoreversion of the *ura3-3* mutation is much more frequent than true reversion, all Ura$^+$ Met$^-$ colonies are assumed to be the result of a recombination event between the mutant *ura3* heteroalleles.

Meiotic analysis of recombination between repeated genes: The meiotic frequencies of Ura$^+$ random spores in strains SJR58 and SJR59 were determined in four independent experiments, and the data are summarized in Table 2. In each experiment, a single colony was inoculated into liquid medium and grown to mid-log. Aliquots of the vegetative cells were plated selectively to determine the frequency of mitotically derived Ura$^+$ segregants, and the remainder of the culture was sporulated. After approximately four days in sporulation medium, random spores were plated selectively to determine the frequency of meiotically derived Ura$^+$ haploid spores. In each experiment, the frequency of meiotic Ura$^+$ colonies is much greater than the frequency of mitotic Ura$^+$ colonies. The meiotic frequency of conversion between the allelic *ura3*$^-$ genes in strain SJR58 is 17-fold greater than that between the same

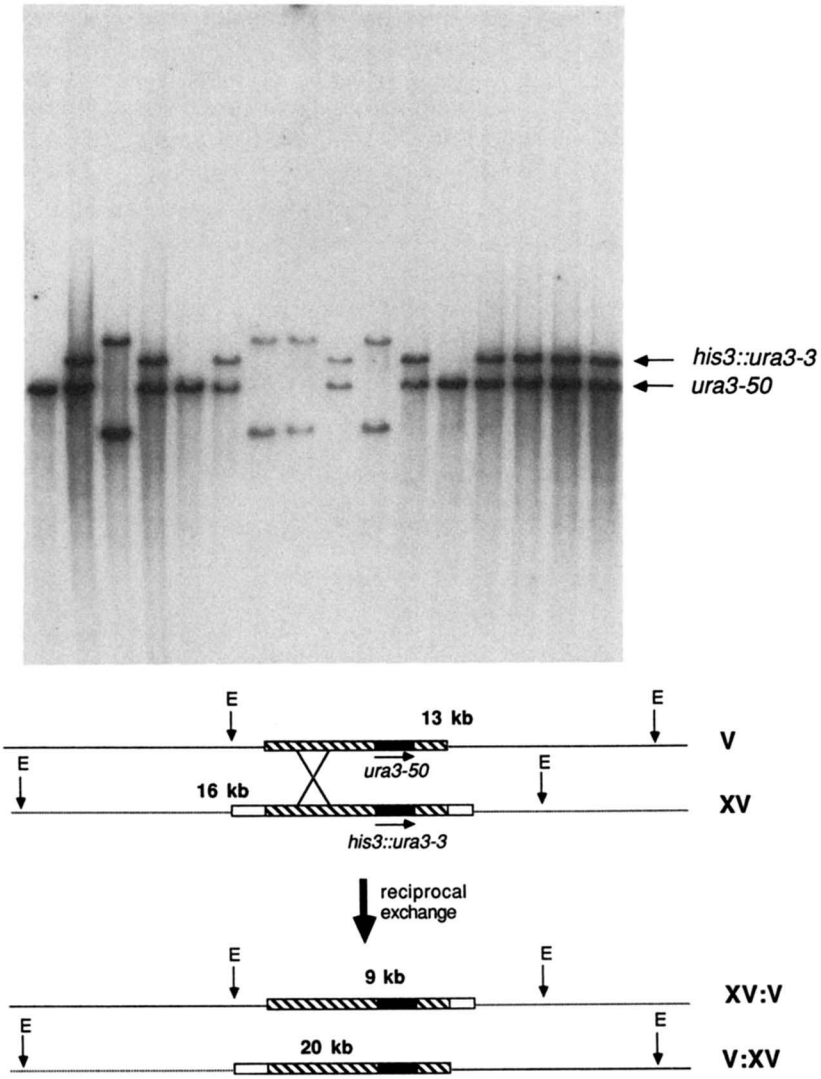

FIGURE 3.—Physical analysis of DNAs from SJR59 Ura$^+$ random spores. Spore DNAs were digested with *Eco*RI and analyzed on a 0.5% agarose gel. Fragments were transferred to nitrocellulose and hybridized to *URA3*-homologous sequences (plasmid pSR13). The *ura3-50* gene on chromosome *V* and the *his3::ura3-3* gene on chromosome *XV* are on fragments of approximately 13 and 16 kb, respectively. A reciprocal exchange event between the mutant *ura3* genes yields fragments of approximately 9 and 20 kb [fragment sizes were calculated from data presented by STRUHL and DAVIS (1980) and ROSE, GRISAFI and BOTSTEIN (1984)]. Since the original diploid was heterozygous for the *ura3* insertion at the *HIS3* locus, some of the nonrecombinant spores have only one *URA3*-homologous fragment. A schematic representation of a reciprocal exchange event between the 5.5-kb *ura3* repeats in strain SJR59 is shown below the Southern blot. The open areas correspond to the 1.7-kb *Bam*HI fragment encoding the *HIS3* gene; the hatched areas correspond to the sequences that flank the *URA3* coding sequence (filled areas) on the 5.5-kb *Bam*HI fragment. The relevant *Eco*RI (E) sites are indicated.

TABLE 2

Meiotic recombination between *ura3*⁻ genes in strains SJR58 and SJR59

Recombination between allelic *ura3*⁻ genes (strain SJR58)				Recombination between *ura3*⁻ genes on nonhomologous chromosomes (strain SJR59)			
	Frequency of Ura⁺ colonies		Frequency of reciprocal recombination		Frequency of Ura⁺ colonies		
Experiment	Mitotic ($\times 10^{-6}$)	Meiotic ($\times 10^{-4}$)	Bgl/Leu (Can/Leu)	Experiment	Mitotic ($\times 10^{-7}$)	Meiotic ($\times 10^{-5}$)	Frequency of reciprocal recombination
1	3.9 ± 0.4	8.6 ± 0.4	0.75 (0.59)	1	6.9 ± 0.9	4.4 ± 0.2	0.26
2	4.8 ± 0.5	9.2 ± 0.6	0.62 (0.51)	2	4.6 ± 0.8	5.8 ± 0.2	0.17
3	12.7 ± 1.5	8.5 ± 0.7	0.70 (0.31)	3	9.5 ±1.9	4.9 ± 0.5	0.09
4	5.4 ± 0.5	7.9 ± 0.5	0.50 (0.38)	4	12.5 ±1.6	4.2 ± 0.5	0.13
Mean		8.5 ± 0.3	0.62 (0.44)	Mean		5.0 ± 0.1	0.16

The mitotic and meiotic frequencies of Ura⁺ colonies in each experiment are given as the mean ± the standard deviation. These values were calculated as detailed in the APPENDIX using selected (Ura⁺) and unselected (total) colony counts from at least three plates. The number of colonies per plate was between 50 and 200, except for the selective platings of the SJR59 mitotic cultures. From these cultures, the number of colonies per plate averaged between five and ten, and colonies on eight plates were counted. The mean meiotic frequency of Ura⁺ colonies in each strain is a weighted mean calculated from the mean and standard deviation of the four independent experiments, as described in the APPENDIX. The frequency of conversion-associated reciprocal exchange of flanking markers in Ura⁺ spores derived from strain SJR58 was calculated for each experiment according to the equation $x = (y - d)/1 - d)$ of PERKINS (1979), where x = the corrected fraction of conversion-associated crossovers; y = the observed fraction of conversion-associated crossovers; and d = the map distance $\times 10^{-2}$ between the flanking markers (incidental exchanges). This equation corrects for incidental exchanges (nonconversion-associated exchanges) in the interval being examined. Instead of using published values for the frequencies of incidental exchanges, the crossover frequency betwen *LEU2* and *CAN1* was measured in unselected SJR58 random spores in each experiment (average crossover frequency = 29%) and that between the *LEU2* and the *Bgl*II polymorphisms was approximated by measuring the *LEU2-URA3* distance by tetrad dissection of strain SJR58 (crossover frequency = 7%). In each experiment with SJR59, the conversion-associated reciprocal exchange values are the observed frequencies of recombination. The mean reciprocal exchange frequency for each strain was calculated by summing the values in the four independent experiments.

mutant genes on nonhomologous chromosomes in strain SJR59. This difference in frequencies is consistent with our previous results using *HIS3* repeats which indicated that the frequencies would differ by, at most, 30-fold (JINKS-ROBERTSON and PETES 1985). It should be noted that determining the frequency of Ura⁺ colonies does not accurately reflect the absolute frequency of conversion between the *ura3*⁻ genes, because only a fraction of the events would be expected to result in a Ura⁺ phenotype. This method should, however, accurately determine relative frequencies of gene conversion. It should also be noted that there are two *ura3-50* genes in strain SJR59, but only one *ura3-50* gene in SJR58 (both strains have one *ura3-3* gene). Although this discrepancy in gene copy number would be expected to increase the relative frequency of interactions between the *ura3*⁻ genes in strain SJR59 by a factor of two, we believe that this increase would be offset by the spore inviability resulting from conversion-associated reciprocal exchanges in strain SJR59 (see below).

From each independent group of random spores, we analyzed 24 Ura$^+$ spores for evidence of reciprocal recombination of flanking markers as described in the preceding section. For the control strain SJR58, reciprocal exchange was monitored using two sets of flanking markers. The average frequency of reciprocal exchange determined using the *LEU2*$^+$ and *Bgl*II polymorphisms is slightly higher than that determined using the *LEU2*$^+$ and *CAN1* markers (62% *vs.* 44%; see Table 2). Regardless of the reason for the slight difference in exchange frequencies, it can be concluded that the frequency of conversion-associated reciprocal exchange in the experiments with the control strain SJR58 is close to 50%.

Physical analysis of Ura$^+$ random spores derived from the experimental strain SJR59 indicates that 16% of the events (15 of 94; see Table 2) leading to a Ura$^+$ phenotype are associated with reciprocal recombination. It should be noted that, in order for a meiotic reciprocal exchange event between nonhomologous chromosomes to be detected, both translocation products must segregate to the same haploid spore. Spores with one of the translocation chromosomes plus one of the normal chromosomes would presumably be inviable since they would lack a complete haploid genome. If one assumes that recombination between the mutant *ura3* genes on chromosomes *V* and *XV* occurs after chromosome replication and that the subsequent segregation of chromatids is completely random, only one-quarter of the exchange events will give rise to viable products (the remaining three-quarters will have one normal chromosome and one of the translocation chromosomes). The exchange value of 16% determined experimentally is, therefore, likely to be an underestimate of the real value. The real exchange value can be estimated by correcting both the number of Ura$^+$ spores showing conversion-associated reciprocal exchange and the total number of Ura$^+$ spores examined for the number of inviable spores generated by conversion-associated reciprocal exchange (three times the number of spores with the reciprocal exchange products). If m Ura$^+$ spores are examined physically for conversion-associated reciprocal exchange and n of these spores have the reciprocal exchange products, then the actual (corrected) frequency of conversion-associated reciprocal exchange is calculated using the equation $(n + 3n)/(m + 3n)$. We thus estimate that the frequency of conversion-associated reciprocal exchange is close to 43%. Based on this revised value, we conclude that conversion between genes on nonhomologous chromosomes is similar to that between allelic genes on homologous chromosomes with respect to associated reciprocal exchange.

The reciprocal translocations between chromosomes *V* and *XV* identified by physical analysis of Ura$^+$ random spores were confirmed by two genetic tests. First, six putative translocation heterozygotes were constructed by mating translocation-bearing spores to normal haploid strains. Dissection of the resulting diploids gave spore viability patterns characteristic of translocation heterozygotes; tetrads segregated predominantly 4 live:0 dead, 2 live:2 dead and 0 live:4 dead spores (PERKINS and BARRY 1977). In 110 tetrads dissected from one such diploid, 18 tetrads segregated 4 live:0 dead, 3 segregated 3 live:1

dead, 71 segregated 2 live:2 dead, 0 segregated 1 live:3 dead and 18 segregated 0 live:4 dead.

In a second genetic test, physical linkage of sequences on chromosomes *V* and *XV* in translocation-bearing spores was inferred by demonstrating mitotic linkage of a locus centromere-distal to the *URA3* locus on chromosome *V* (*CAN1*) to a locus centromere-proximal to the *HIS3* locus on chromosome *XV* (*ADE2*). *ADE2*$^+$ strains are adenine prototrophs and colonies are white in color; *ade2*$^-$ strains require adenine for growth and are red in color due to the accumulation of a pigmented precursor to adenine. For the mitotic linkage analysis, a translocation homozygote (strain SJR64) was constructed by mating two of the translocation-bearing Ura$^+$ random spores. One spore was *ade2*$^-$*can1*^{r} and the other was *ADE2*$^+$*CAN1*^{s}, so that the resulting diploid was heterozygous at both relevant loci, but was phenotypically Ade$^+$Can^s (the wild-type allele is dominant at each locus). Single cells of strain SJR64 were briefly irradiated with ultraviolet light to induce mitotic recombination, and these cells were grown to colonies on nonselective medium. If an induced mitotic recombination event occurred between the centromere and the *ADE2* locus, then the cell would be expected to give rise to an *ADE2*$^+$/*ADE2*$^+$ cell plus an *ade2*$^-$/*ade2*$^-$ cell 50% of the time (see Figure 4 for an analogous event involving the *CAN1* locus). The resulting colony would appear sectored for the Ade phenotype; half of the colony would be red, Ade$^-$ and the other half white, Ade$^+$. If the *CAN1* locus is centromere-distal to the *ADE2* locus on the chromosome where the recombination event occurred, the *CAN1* alleles should likewise be homozygous in each sector of the colony; the Ade$^-$ sector should thus be Can^r and the Ade$^+$ sector be Can^s. In an experiment with strain SJR64, approximately 2% (33 of 1930) of the irradiated cells gave rise to red/white sectored colonies, and 80% (27 of 33) of these colonies were also sectored Can^r/Can^s. Each sector of one such colony was sporulated, and subsequent tetrad analysis confirmed that each sector was homozygous at the *ADE2* and *CAN1* loci.

The location of the *URA3*$^+$ gene in 23 of the nontranslocation bearing Ura$^+$ spores derived from strain SJR59 was determined by mating the spores with appropriate *URA3*$^+$ tester haploids. A 4+:0− segregation pattern upon sporulation of a given diploid would indicate that the *URA3*$^+$ gene in the spore is allelic to that in the tester haploid and is therefore at the *URA3* locus on chromosome *V*. A departure from the 4+:0− segregation pattern would indicate that the *URA3*$^+$ gene in the spore is not at the *URA3* locus; it would presumably be at the *HIS3* locus on chromosome *XV*. In 26 spores examined, 17 had the *URA3*$^+$ at the *URA3* locus, and the remaining nine were presumed to have the *URA3*$^+$ gene at the *HIS3* locus. Chi square analysis of this data indicates that these numbers are not significantly different from the equal numbers expected if there is no bias in the direction of conversion ($\chi^2 = 1.88$; $0.2 < P < 0.1$). The location of the *URA3*$^+$ gene in nine of the translocation-bearing spores was also determined. In eight of these spores, the *URA3*$^+$ gene was linked to the centromere of chromosome *XV*; in one spore, the *URA3*$^+$ gene was linked to the centromere of chromosome *V*. This bias in the location

of the *URA3*$^+$ gene is the one expected, given the relative positions of the *ura3-3* and *ura3-50* mutations within the *URA3* coding sequence.

Mitotic analysis of recombination between repeated genes: The mitotic rate of gene conversion between the mutant *ura3* genes in strains SJR58 and SJR59 was calculated as described by LEA and COULSON (1948), using an experimentally determined median frequency of gene conversion. It should be noted that the conversion rates thus determined are smaller than the measured frequencies because the calculation corrects for amplification (by cell division) of events that occur before the time the frequency was measured (in meiotic experiments no correction is necessary, because mitotic divisions do not occur between meiosis and the time of frequency measurement). For each strain, the median frequency of conversion between the mutant *ura3* genes was found by measuring the frequency of Ura$^+$ colonies in 20 independent cultures (Table 3). The conversion rate between the mutant *ura3* genes was found to be fivefold greater when they are at allelic positions on homologous chromosomes in strain SJR58 (8.1×10^{-7}) than when they are on nonhomologous chromosomes in strain SJR59 (1.7×10^{-7}). A difference in recombination rates would not necessarily be expected, because there is assumed to be no pairing of homologous chromosomes in mitosis to facilitate interactions. The rate differences observed may simply reflect the effect of absolute sequence homology around the *ura3*$^-$ genes on recombination (all of chromosome *V* in SJR58 *vs.* 5.5 kb in SJR59), or could reflect the positioning of specific chromosomes within the nucleus.

In addition to mitotic recombination rate determinations, we also measured the frequency of conversion-associated recombination of flanking markers in mitosis. Approximately 100 independent Ura$^+$ segregants from each strain were analyzed for conversion-associated reciprocal exchange. In strain SJR58, heterozygosity at the centromere-distal *CAN1* locus was used to monitor reciprocal exchange associated with conversion between the centromere-proximal *ura3* heteroalleles at the *URA3* locus. As illustrated in Figure 4, a mitotic reciprocal exchange between the centromere of chromosome *V* and the heterozygous *CAN1* locus will yield two chromosome *V* homologues in which one sister chromatid carries the wild-type *CAN1*^{s} allele and the other sister carries the mutant *can1*^{r} allele. The segregation of two nonsister chromatids into the mitotic progeny will yield either two cells that are heterozygous *CAN1*^{s}/*can1*^{r} or one cell homozygous for the *CAN1*^{s} allele and one homozygous for the *can1*^{r} allele. Since the wild-type *CAN1*^{s} allele is dominant to the mutant *can1*^{r} allele, heterozygotes are phenotypically sensitive to canavanine. A cell heterozygous at *CAN1* will give rise to Can^r progeny by recombination or chromosome loss, and these progeny can be readily visualized on selective medium as small Can^r colonies growing against a background of Can^s cells. A homozygous diploid *CAN1*^{s} strain can be distinguished from a heterozygous strain because the former will virtually never produce Can^r colonies, since both wild-type copies of the gene must mutate in order to get a Can^r phenotype. A homozygous *can1*^{r} strain will be resistant to canavanine. It is thus possible to determine visually the genotype at the *CAN1* locus. If it is assumed that chromatid

TABLE 3

Mitotic recombination between *ura3*⁻ genes in strains SJR58 and SJR59

Strain SJR58			Strain SJR59		
Culture no.	Total cells plated ($\times 10^7$)	Total Ura$^+$ colonies	Culture no.	Total cells plated ($\times 10^7$)	Total Ura$^+$ colonies
1	5.6	178	1	5.9	60
2	4.9	203	2	7.5	61
3	4.1	140	3	7.4	60
4	4.0	218	4	8.2	65
5	5.4	155	5	8.7	79
6	4.0	158	6	8.8	60
7	4.8	294	7	11.7	58
8	5.8	314	8	10.7	76
9	5.3	157	9	10.8	61
10	5.5	177	10	8.1	79
11	5.9	328	11	15.5	104
12	5.4	239	12	16.9	118
13	5.9	292	13	14.0	128
14	6.4	767	14	15.4	85
15	7.4	242	15	16.2	143
16	4.2	387	16	14.6	98
17	5.8	134	17	11.4	113
18	3.9	141	18	13.1	128
19	6.8	222	19	13.4	205
20	7.8	441	20	14.0	292
Average no. of cells/culture					
5.4×10^7				11.6×10^7	
Median no. of Ura$^+$ colonies/culture					
220				82	
Conversion rate/generation $\times 10^{-7}$					
8.1 ± 1.1^a				1.7 ± 0.3	
Frequency of conversion-associated reciprocal exchange					
10%				6%	

[a] Conversion rate ± standard deviation.

segregation is random in mitosis, only one-half of the mitotic recombination events will yield homozygous progeny. The frequency of recombination is, therefore, two times the observed number of homozygous cells among Ura$^+$ convertants. Three homozygous *CAN1*^{s} and two homozygous *can1*^{r} convertants were detected in 99 Ura$^+$ colonies derived from SJR58. Thus, about 10% of the mitotic conversion events between the *ura3* heteroalleles were associated with reciprocal recombination of the flanking DNA.

For the mitotic Ura$^+$ segregants of SJR59, conversion-associated reciprocal exchange was detected as described for the meiotic experiments. DNA was isolated from Ura$^+$ colonies and examined by Southern blot analysis for the appearance of a fragment or fragments diagnostic of a reciprocal translocation between chromosomes *V* and *XV* (see Figure 3). This procedure, in contrast

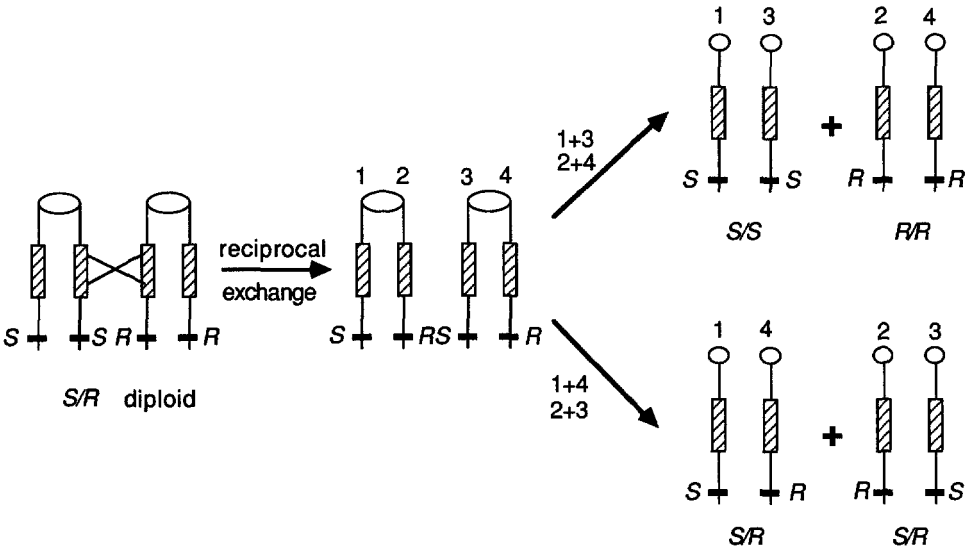

FIGURE 4.—Homozygosis of *CAN1* alleles in a diploid strain heterozygous at the *CAN1* locus. On the left, chromosome *V* homologues are shown after DNA replication when sister chromatids are attached at their centromeres. The hatched rectangles represent the *URA3* locus, and the alleles present at the distal *CAN1* locus are indicated by S or R. S corresponds to the wild-type, canavanine-sensitive allele; R corresponds to the mutant, canavanine-resistant allele. Chromatids are numbered 1–4. Chromatids *1* and *2* always segregate away from one another, as do chromatids *3* and *4*.

to that using the heterozygous *CAN1* locus in strain SJR58, should detect all conversion-associated reciprocal exchanges in strain SJR59. Three Ura$^+$ convertants out of 93 analyzed had one of the translocation fragments, and three convertants had both; this translates to a reciprocal exchange frequency of 6%. This result confirms previous reports that mitotic recombination between repeated genes on nonhomologous chromosomes can produce chromosomal translocations (MIKUS and PETES 1982; POTIER, WINSOR and LACROUTE 1982; SUGAWARA and SZOSTAK 1983). Translocations resulting from interactions between dispersed Ty elements have also been detected by selecting for modification of Ty elements located in front of selectable genes (CHALEFF and FINK 1980; BRIELMANN, GAFNER and CIRIACY 1985).

The mitotic data obtained with strains SJR58 and SJR59 are summarized and compared with the meiotic data in Table 4. The rate of conversion per division between the mutant *ura3* genes, as inferred by measuring the frequency of Ura$^+$ colonies, is 1000- and 300-fold higher in meiosis than in mitosis in strains SJR58 and SJR59, respectively. Similarly, in both SJR58 and SJR59, the frequency of conversion-associated reciprocal exchange is higher in meiosis than in mitosis by about a factor of five. This latter observation probably reflects a basic mechanistic difference between meiotic and mitotic recombination in yeast. Meiotic recombination, for example, occurs in G_2 after DNA replication whereas mitotic recombination may be either a G_1 or G_2 event (ESPOSITO and WAGSTAFF 1981; ROMAN and FABRE 1983). In this regard, it

TABLE 4

Summary of recombination between *ura3*$^-$ genes in strains SJR58 and SJR59

	Conversion rate per generation	Conversion-associated reciprocal exchange (%)
SJR58 mitotic	8.1×10^{-7}	10
SJR58 meiotic	8.6×10^{-4}	53
SJR59 mitotic	1.7×10^{-7}	6
SJR59 meiotic	5.2×10^{-5}	43

should also be noted that several recombination mutants have been isolated that affect meiotic and mitotic recombination differently (see ORR-WEAVER and SZOSTAK 1985).

DISCUSSION

Isogenic diploid strains were constructed with two different mutant *ura3* genes at either allelic positions on homologous chromosomes or on nonhomologous chromosomes. Meiotic and mitotic gene conversion events between the *ura3*$^-$ genes were detected by selecting for Ura$^+$ colonies, and these colonies were subsequently examined for conversion-associated recombination of flanking markers. The recombination data obtained from these experiments are summarized in Table 4. We have shown that gene conversion and reciprocal recombination between repeated sequences on nonhomologous chromosomes occurs at a high frequency during meiosis. In the present study, the frequency of such events is 17-fold less than the frequency of normal allelic gene conversion for the same sequences. In a previous study using *HIS3* repeats in different genomic locations (JINKS-ROBERTSON and PETES 1985), we found only a threefold difference between allelic conversion and conversion between the repeated sequences on nonhomologous chromosomes. In addition, M. LICHTEN, R. BORTS and J. HABER (personal communication) have obtained results similar to ours using a different pair of interacting yeast genes. Since the difference in the relative meiotic recombination frequencies between sequences on homologous *vs.* nonhomologous chromosomes in these studies is unexpectedly small, we suggest that the characteristic end-to-end pairing (synapsis) of homologous chromosomes that occurs in meiosis is not an absolute prerequisite for high levels of recombination. In yeast, sequence homology rather than chromosome synapsis *per se* seems to be all that is required to promote meiotic recombination. The observation that the ratio of meiotic to mitotic gene conversion is similar when the interacting genes are either on homologous chromosomes or on nonhomologous chromosomes (see Table 4) provides further support for the notion that chromosome synapsis alone does not adequately account for the high levels of meiotic recombination.

Meiotic chromosome synapsis is usually equated with formation between paired homologues of the tripartite structural element known as the synaptonemal complex, and it is generally assumed that the complex plays a critical

role in recombination (VON WETTSTEIN, RASMUSSEN and HOLM 1984). Our results indicate either that synaptonemal complexes are not required for high levels of meiotic recombination or that complexes can efficiently form (at least transiently) between repeated sequences on nonhomologous chromosomes. Regardless of which of these possibilities is correct, the role of the synaptonemal complex in meiosis is different from that normally accepted and deserves reexamination. If the first possibility is true, the complex is likely to have a function that is not directly related to recombination; if the second possibility is correct, synaptonemal complex formation is related to DNA sequence homology, rather than being the result of an interaction of homologous chromosomes. We favor the first possibility since in normal diploid meioses in yeast (BYERS and GOETSCH 1975), as well as in other organisms (VON WETTSTEIN, RASMUSSEN and HOLM 1984), synaptonemal complexes do not involve nonhomologous chromosomes. It should also be noted that attempts to identify synaptonemal complexes in the yeast *Schizosaccharomyces pombe* have been unsuccessful (OLSON *et al.* 1978), and yet this yeast has levels of meiotic recombination similar to those observed in *S. cerevisiae*.

The 17-fold difference in the meiotic frequencies of gene conversion between allelic sequences and the same sequences on nonhomologous chromosomes could be attributed to several factors. While some of the meiotic difference in frequencies could be attributed to chromosome synapsis and the formation of synaptonemal complexes between the allelic sequences, the observation of a fivefold difference in mitotic frequencies indicates that factors other than meiotic-specific chromosome synapsis and synaptonemal complex formation must be important. The difference in conversion frequencies in both mitosis and meiosis could, for example, simply reflect the extent of sequence homology flanking the interacting mutant *ura3* genes when they are on homologous *vs.* nonhomologous chromosomes. Alternatively, it may be that chromosomes are arranged within the nucleus, such that sequences on homologous chromosomes are closer together (and, therefore, more likely to recombine) than sequences on nonhomologous chromosomes.

Allelic meiotic conversion events are associated with reciprocal recombination of flanking markers about 50% of the time (FOGEL, MORTIMER and LUSNAK 1981). In the experiments described here, we have demonstrated that a similar level of conversion-associated reciprocal exchange (approximately 50%) occurs when the interacting *ura3*$^-$ genes are on either homologous or nonhomologous chromosomes. The high frequency of meiotic gene conversion between the repeated sequences on nonhomologous chromosomes is thus associated with the formation of chromosome rearrangements, specifically reciprocal translocations. Two interesting conclusions can be drawn from this observation. First, the conversion events between the repeats on nonhomologous chromosomes are the result of direct chromosome-chromosome interactions. Although this does not exclude the possibility that some gene conversion events between dispersed repeats may be mediated by a diffusable RNA or DNA intermediate (MORZYCKA-WROBLEWSKA *et al.* 1985; JINKS-ROBERTSON and PETES 1985), our observations make such models less likely. Second, our results

suggest that meiotic recombination between dispersed repeats may be an important mechanism for generating spontaneous chromosomal translocations, as well as other types of chromosome rearrangements (inversions, deletions, duplications). Since chromosomal rearrangements have been implicated in the formation and maintenance of species (DOBZHANSKY 1937), we believe that interactions between dispersed repeats are likely to be important in evolution. In addition, the conversion events that occur in the absence of reciprocal recombination are likely to be an important factor in maintaining sequence homogeneity among families of dispersed repeats (EDELMAN and GALLY 1970).

The results presented here using an artificially constructed *URA3* duplication raise an interesting paradox if one assumes that pairing is the limiting step in recombination between naturally occurring repeated sequences. There are, for example, approximately 70 copies of the 6-kb transposable element Ty present in a diploid yeast genome. If one considers the number of possible pairwise interactions between the Ty elements (2400 possible pairwise interactions), and assumes that the frequency of interaction measured between the *ura3*$^-$ repeats (2×10^{-4} per tetrad) is relevant to that occurring between a single pair of Ty repeats, then we would predict that there should be very frequent conversion between Ty elements. If conversion events between Ty elements were associated with reciprocal recombination, one would expect to frequently observe chromosome rearrangements (deletions, duplications, translocations, dicentrics) in yeast. Although there are no accurate measurements of the frequency of such alterations in chromosome structure, spore viability data indicate that they are much less frequent than our simple calculation would predict. We suggest, therefore, that (1) interactions between the naturally occurring Ty elements are specifically repressed; (2) the interactions are resolved almost exclusively as noncrossovers; or (3) the frequency of meiotic interactions is greatly reduced by the sequence heterology observed among different Ty elements. If one assumes that the limiting factor in recombination between repeated sequences is the initiating event rather than pairing between the elements, then we would estimate that the frequency of recombination between Ty elements should be approximately 35 times that observed for the single pair of *ura3*$^-$ repeats. If this assumption is correct, one need not postulate a recombination-priviledged state for Ty elements.

Meiotic experiments similar to those described here have been done in the fission yeast *S. pombe* using dispersed serine tRNA genes as repeats (AMSTUTZ *et al.* 1985). While the conversion frequencies observed in the *S. pombe* experiments are similar to those reported here, conversion events between tRNA genes on nonhomologous chromosomes are virtually never associated with reciprocal recombination (KOHLI *et al.* 1984). Although the reason for the huge difference in the level of conversion-associated reciprocal exchange is not clear, one possibility is that the small size of the tRNA repeats (200 bp) might preclude the resolution of interactions as reciprocal exchange events (KOHLI *et al.* 1984; see also KLAR and STRATHERN 1984; CARPENTER 1984).

Previous experiments in yeast have indicated that the frequency of recombination between allelic sequences is several orders of magnitude higher in

meiosis than in mitosis (ESPOSITO and WAGSTAFF 1981). In our experiments with the *ura3* heteroalleles, the frequency of meiotic recombination events was two to three orders of magnitude greater than the frequency of analogous mitotic events when the genes were on either homologous or nonhomologous chromosomes. As expected from previous studies (reviewed by ORR-WEAVER and SZOSTAK 1985), we found that the mitotic conversion events, like meiotic conversion events, were associated with reciprocal recombination of flanking markers. This association, however, for both allelic recombination and recombination between repeats on nonhomologous chromosomes, was weaker than that observed in meiosis (50% associated reciprocal exchanges in meiosis *vs.* only 10% in mitosis). Since the frequency of mitotic interactions between dispersed repeats is much lower than the analogous meiotic events, we believe it is likely that most spontaneous chromosomal translocations (and other rearrangements) generated by homologous recombination arise during meiosis.

Chromosomal rearrangements have been observed in essentially all organisms that have been well characterized genetically. Although the origins of the rearrangements are not known, it is likely that at least some may be the result of homologous recombination between dispersed repeats similar to that we have characterized in yeast. Studies on recombination between dispersed repeats in a genetically amenable organism such as yeast should thus be applicable to issues of recombination and genome evolution in higher eukaryotes. To distinguish recombination between repeats on nonhomologous chromosomes from other types of recombination (recombination between repeats within a chromosome, recombination between sequences on homologous chromosomes, etc.), we propose that the term "heterochromosomal" recombination be used to describe this type of event.

We thank T. NAGYLAKI, R. R. BAHADUR, P. MEIER and S. L. ZABELL for helpful discussions concerning statistical analyses of the data; D. D. PERKINS for comments on correcting meiotic conversion-associated reciprocal exchange values for incidental exchanges; M. LICHTEN for the equation calculating the reciprocal exchange frequency between repeats on nonhomologous chromosomes; and S. C. FALCO for providing some of the plasmids and strains used in this study. We also thank members of this laboratory for helpful discussions throughout the course of this work, and for reading and commenting on the manuscript. This work was supported by National Institutes of Health grant GM24110-09 to T.D.P. and National Institutes of Health postdoctoral fellowship GM09628-03 to S.J.-R.

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Communicating editor: D. BOTSTEIN

APPENDIX

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Here, we outline the method of estimating the meiotic conversion rates in Table 2. Our approach takes into account that the accuracy of the conversion rates estimated from different cultures may vary significantly.

From each of n cultures, we draw k and m independent samples to estimate the number of recombinant cells and the total number of cells, respectively. Let x_{ij} ($i = 1, \cdots, n$; $j = 1, \cdots, k$) denote the number of recombinant cells in the ith culture, estimated from the jth sample, and define the means and variances

$$\bar{x}_i = \frac{1}{k}\sum_{j=1}^{k} x_{ij}, \tag{1a}$$

$$s^2_{\bar{x}_i} = \frac{1}{k(k-1)}\sum_{j=1}^{k} (x_{ij} - \bar{x}_i)^2. \tag{1b}$$

Let y_{ij} ($i = 1, \cdots, n$; $j = 1, \cdots, m$) denote the total number of cells in the ith culture, estimated from the jth sample, and put

$$\bar{y}_i = \frac{1}{m}\sum_{j=1}^{m} y_{ij}, \tag{2a}$$

$$s^2_{\bar{y}_i} = \frac{1}{m(m-1)}\sum_{j=1}^{m} (y_{ij} - \bar{y}_i)^2. \tag{2b}$$

The exact conversion rate in the ith culture is the ratio of expectations:

$$p_i = E(\bar{x}_i)/E(\bar{y}_i). \tag{3}$$

If $s_{\bar{y}_i} \ll \bar{y}_i$, we may estimate p_i and its variance from the approximations

$$p_i \approx \bar{x}_i/\bar{y}_i, \tag{4a}$$

$$s^2_{p_i} \approx p_i^2[(s_{\bar{x}_i}/\bar{x}_i)^2 + (s_{\bar{y}_i}/\bar{y}_i)^2]. \tag{4b}$$

We combine the estimates p_i from different cultures by weighting with the reciprocals of their variances. Thus, setting

$$a_i = 1/s^2_{p_i}, \qquad b_i = a_i \Big/ \sum_{j=1}^{n} a_j, \tag{5}$$

we have

$$\bar{p} = \sum_{i=1}^{n} b_i p_i, \tag{6a}$$

$$s^2_{\bar{p}} = 1 \Big/ \sum_{i=1}^{n} a_i. \tag{6b}$$