

# High-frequency meiotic gene conversion between repeated genes on nonhomologous chromosomes in yeast

(recombination/synaptonemal complex/concerted evolution)

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**ABSTRACT** We have used a genetic system that allows detection of meiotic recombination events between repeated sequences on nonhomologous chromosomes in the yeast *Saccharomyces cerevisiae*. We have found that recombination between these sequences occurs at a frequency of about 0.5%, and the events observed were nonreciprocal (gene conversions). Surprisingly, the frequency of conversion between the repeated genes on nonhomologous chromosomes observed in this study is similar to that observed between allelic genes. This result is discussed in connection with the role of the synaptonemal complex in meiotic recombination and with the relationship between reciprocal and nonreciprocal recombination.

Genetic recombination between homologous sequences in eukaryotic organisms occurs in meiosis and mitosis and may be either a reciprocal or nonreciprocal (gene conversion) event. Recombination is usually thought of as involving allelic sequences, but it may also occur between repeated genes. For purposes of this discussion, we define "alleles" as genes located at the same position on homologous chromosomes. The term "repeated genes" will refer to genes that are present in more than one copy per haploid genome. In the yeast *Saccharomyces cerevisiae*, both mitotic and meiotic recombination have been observed between repeated genes located on the same chromosome or on homologous chromosomes (1-9). In contrast, only mitotic recombination has been observed when the repeated genes are located on nonhomologous chromosomes (10-14). In the fission yeast *Schizosaccharomyces pombe*, low levels of both meiotic and mitotic recombination between repeated tRNA genes on nonhomologous chromosomes have been demonstrated (15).

We have developed a genetic system that is designed to detect unusual genetic events occurring during meiosis in *S. cerevisiae*. Using this system, we show that gene conversion (nonreciprocal transfer of information) between repeated genes on nonhomologous chromosomes occurs frequently during meiosis in yeast. This frequency is similar to that observed when the same genes are at allelic positions. In addition, we find that the meiotic frequency of gene conversion between repeated genes on nonhomologous chromosomes is significantly higher than the mitotic frequency.

## MATERIALS AND METHODS

**Plasmid Constructions.** Standard techniques were used for the restriction, ligation, and analysis of plasmid DNAs (16). The structures of plasmids pRB58, pSR4, and pSR5 are shown in Fig. 1. Plasmid pSR4 was constructed by inserting a 1.75-kilobase (kb) *Bam*HI fragment containing the *HIS3*<sup>+</sup> gene (from YEp6; ref. 18) into the unique *Bam*HI site within the *SUC2*<sup>+</sup> gene on plasmid pRB58. To construct plasmid

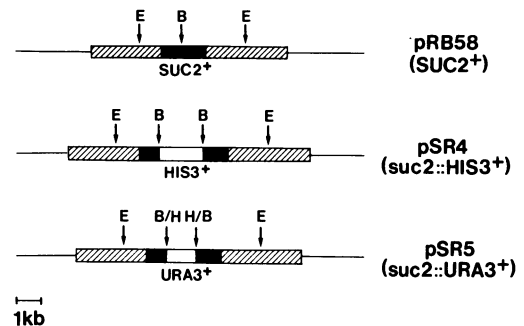


FIG. 1. Structures of plasmids pRB58, pSR4, and pSR5. Boxes represent yeast DNA and lines represent YEp24 vector sequences. For simplicity, only the relevant features of the plasmids are shown. Plasmid pRB58 was constructed and characterized by M. Carlson (see ref. 17 for similar constructions). This plasmid is a YEp24 derivative and contains a 7.9-kb insert of yeast DNA encoding the *SUC2*<sup>+</sup> gene and flanking sequences. The plasmid YEp24 (17) is a yeast-*Escherichia coli* shuttle vector that contains pBR322 sequences, 2- $\mu$ m DNA, and the *URA3*<sup>+</sup> gene. Plasmid pSR4 was constructed by inserting a 1.75-kb *Bam*HI fragment containing the yeast *HIS3*<sup>+</sup> gene into the unique *Bam*HI site within the *SUC2*<sup>+</sup> gene on pRB58. Plasmid pSR5 was constructed by inserting a 1.2-kb *Hind*III fragment containing the yeast *URA3*<sup>+</sup> gene into the *Bam*HI site of pRB58 after filling in the enzyme-generated cohesive ends with DNA polymerase. Only the relevant *Bam*HI (B), *Eco*RI (E), and *Hind*III (H) restriction sites are indicated.

pSR5, we first purified a 1.2-kb *Hind*III fragment containing the *URA3*<sup>+</sup> gene (from YIp30; ref. 18); the cohesive ends generated by the restriction enzyme were filled in using the Klenow fragment of DNA polymerase I. The plasmid pRB58 was digested with *Bam*HI and, following a similar filling in of the *Bam*HI-generated cohesive ends, the *URA3*<sup>+</sup> gene fragment and plasmid were ligated together. By analogy with the bacterial transposon nomenclature, the inactivated *SUC2* genes on plasmids pSR4 and pSR5 are called *suc2*::*HIS3*<sup>+</sup> and *suc2*::*URA3*<sup>+</sup>, respectively. Plasmid pSR7 contains the yeast *HIS3*<sup>+</sup> gene and was constructed by inserting the 1.75-kb *Bam*HI fragment from YEp6 into the *Bam*HI site of pBR322.

**Yeast Strains.** Yeast strains were grown at 30°C and sporulated at room temperature. YEPD medium was used for nonselective growth and synthetic SD media were used for selective growth (19). Standard procedures for mating, sporulating, and dissecting yeast strains were used to construct the haploid strains SJR14 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 1 SUC2<sup>+</sup> leu2-3,112 can1<sup>r</sup>*) and SJR21 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 1 SUC2<sup>+</sup> LEU2<sup>+</sup> CAN1<sup>s</sup>*). The *his3 $\Delta$ 1* allele was constructed *in vitro* by deleting the 150-base-pair *Hind*III fragment from the coding sequence of the yeast *HIS3*<sup>+</sup> gene (20). The *ura3-52* allele has an insertion of the yeast transposable element Ty in the *URA3* coding sequence (21).

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Abbreviation: kb, kilobase(s).

A one-step gene replacement technique (22) was used to replace the *SUC2*<sup>+</sup> alleles in SJR14 and SJR21 with one of the mutant *SUC2* alleles shown in Fig. 1. Strains were transformed as described by Sherman *et al.* (19) with 10 μg of *EcoRI*-digested pSR5 or pSR4. Transformation of yeast with linear DNA fragments stimulates recombination at the free ends, resulting in replacement of the corresponding chromosomal segment (23). Transformants were selected by plating spheroplasts on SD complete medium lacking either histidine or uracil and were subsequently screened for a *Suc*<sup>-</sup> phenotype (see ref. 17). The gene replacements were confirmed by Southern analysis of genomic DNAs from *His*<sup>+</sup>*Suc*<sup>-</sup> and *Ura*<sup>+</sup>*Suc*<sup>-</sup> transformants of SJR21 and SJR14, respectively. Strain SJR22 is identical to SJR14 except for the replacement of the *SUC2*<sup>+</sup> allele with the mutant *suc2::URA3*<sup>+</sup> allele from plasmid pSR5. SJR23 is identical to SJR21 except for the replacement of the *SUC2*<sup>+</sup> allele with the mutant *suc2::HIS3*<sup>+</sup> allele from pSR4. The diploid strain SJR24 was constructed by mating strains SJR22 and SJR23.

The haploid strain SJR32 (*MATa ura3-52 HIS3*<sup>+</sup> *LEU2*<sup>+</sup> *SUC2*<sup>+</sup> *CAN1*<sup>s</sup>) was constructed by transforming strain SJR21 with *Bam*HI-digested pSR7 and selecting *His*<sup>+</sup> transformants. Except for the location of the *HIS3*<sup>+</sup> gene, strain SJR32 is identical to strain SJR23. Strain SJR32 was mated with strain SJR22 to construct the diploid strain SJR33.

## RESULTS

The diploid strain SJR24 (*MATa/MATa ura3-52/ura3-52 his3Δ1/his3Δ1 leu2-3,112/LEU2*<sup>+</sup> *can1<sup>r</sup>/CAN1*<sup>s</sup> *suc2::URA3*<sup>+</sup>/*suc2::HIS3*<sup>+</sup>) has the selectable yeast *HIS3*<sup>+</sup> and *URA3*<sup>+</sup> genes inserted at identical positions within the *SUC2* locus and was constructed as follows. The *suc2::URA3*<sup>+</sup> and *suc2::HIS3*<sup>+</sup> alleles (see Fig. 1) were constructed *in vitro* and transformed into appropriate haploid strains in order to replace the *SUC2*<sup>+</sup> allele with one of the inactivated genes. The haploid strains thus constructed were mated to give the diploid strain SJR24 with the *HIS3*<sup>+</sup> and *URA3*<sup>+</sup> genes at allelic positions. Other details of the constructions are given in *Materials and Methods*.

The relevant features of the SJR24 genotype are schematically shown in Fig. 2. The *SUC2* locus is on chromosome IX, and the diploid strain is heterozygous for the *suc2::HIS3*<sup>+</sup>, *suc2::URA3*<sup>+</sup> alleles. The *URA3* locus on chromosome V and the *HIS3* locus on chromosome XV are homozygous for the nonreverting *ura3-52* and *his3Δ1* mutant alleles, respectively. It should be noted that the *ura3-52* and *his3Δ1* alleles are physically as well as genetically distinguishable from the wild-type alleles at the *SUC2* locus because of an insertion and deletion, respectively (see *Materials and Methods*). The strain SJR24 can thus be used to look for recombination events between the mutant genes at the normal chromosomal locations and the wild-type genes at the *SUC2* locus on chromosome IX.

The expected meiotic segregation patterns of chromosomes V, IX, and XV in strain SJR24 are shown in Fig. 2A. Since each haploid meiotic product should receive exactly one copy of each chromosome, each spore should have the *ura3-52* allele on chromosome V, the *his3Δ1* allele on chromosome XV, and either the *suc2::URA3*<sup>+</sup> or *suc2::HIS3*<sup>+</sup> allele on chromosome IX. Within a tetrad, two spores should have the *suc2::URA3*<sup>+</sup> allele and the other two spores should have the *suc2::HIS3*<sup>+</sup> allele. Therefore, most tetrads should segregate 2 *His*<sup>+</sup>*Ura*<sup>-</sup>:2 *His*<sup>-</sup>*Ura*<sup>+</sup> spores. If a gene conversion event occurs between the allelic *suc2::URA3*<sup>+</sup> and *suc2::HIS3*<sup>+</sup> genes, a 3:1 segregation pattern is expected. A conversion of *suc2::URA3*<sup>+</sup> to *suc2::HIS3*<sup>+</sup>, for example, results in a tetrad with three *His*<sup>+</sup>*Ura*<sup>-</sup> spores and one *His*<sup>-</sup>*Ura*<sup>+</sup> spore as illustrated in Fig. 2B. It is important to note that even when there is a conversion between the mutant

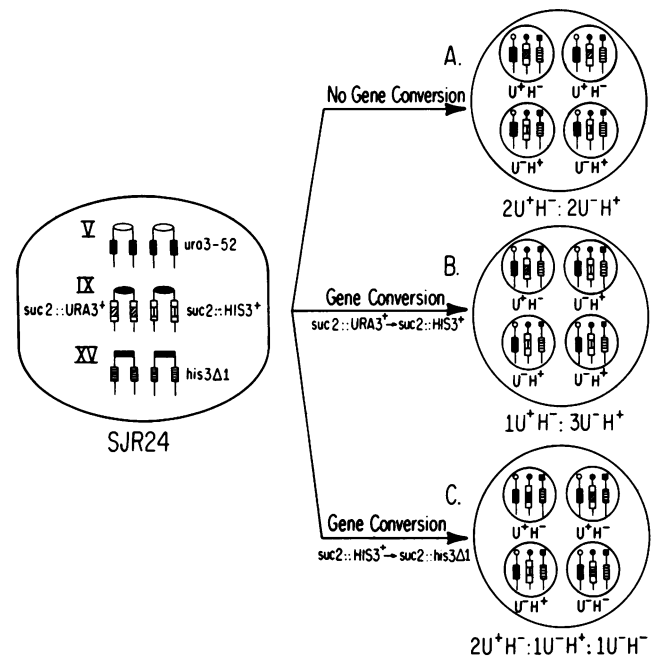


FIG. 2. Meiotic segregation of chromosomes V, IX, and XV in strain SJR24. Chromosomes V, IX, and XV contain the *URA3*, *SUC2*, and *HIS3* loci, respectively, and can be distinguished by their centromeres (○, ●, ■). The diploid SJR24 is shown schematically on the left at a stage preceding the first meiotic division; chromosomes have replicated (sister chromatids are held together at the centromeres) and homologous chromosomes have paired. The four haploid meiotic products expected from no conversion at the *SUC2* locus (A), allelic conversion of *suc2::URA3*<sup>+</sup> to *suc2::HIS3*<sup>+</sup> (B), and nonallelic conversion of *suc2::HIS3*<sup>+</sup> to *suc2::his3Δ1* (C) are shown on the right. The phenotypes of the spores within the 3 tetrads are indicated by U (Ura) and H (His). ●, *ura3-52*; ○, *suc2::URA3*<sup>+</sup>; ■, *suc2::HIS3*<sup>+</sup>; ◐, *his3Δ1*; ◑, *suc2::his3Δ1*.

*suc2* genes, the *suc2::URA3*<sup>+</sup> and *suc2::HIS3*<sup>+</sup> alleles should always segregate away from each other. Each spore should be either *His*<sup>+</sup>*Ura*<sup>-</sup> or *His*<sup>-</sup>*Ura*<sup>+</sup>, but not *His*<sup>+</sup>*Ura*<sup>+</sup> or *His*<sup>-</sup>*Ura*<sup>-</sup>.

Strain SJR24 was sporulated and tetrads were dissected in order to look for aberrant segregations of the *HIS3*<sup>+</sup> and *URA3*<sup>+</sup> genes at the *SUC2* locus. After 3 days of nonselective growth, spores were replica-plated directly onto selective plates to score the segregations of the heterozygous markers. Ninety-six percent of the spores were viable, and a total of 606 tetrads with four viable spores was analyzed. Ninety-five percent of the tetrads showed 2:2 segregation for all markers, and most of the remaining tetrads had a 3:1 segregation pattern at one of the loci examined, indicating a gene conversion. There were 16 conversions at the *LEU2* locus, 4 at *MAT*, and 1 at *CAN1*. One example of postmeiotic segregation was seen at the *CAN1* locus.

Five hundred ninety-six of the 606 tetrads analyzed had the expected segregation pattern of 2 *His*<sup>+</sup>*Ura*<sup>-</sup>:2 *His*<sup>-</sup>*Ura*<sup>+</sup> spores. Seven tetrads had a segregation pattern indicating gene conversion between the *suc2::HIS3*<sup>+</sup> and *suc2::URA3*<sup>+</sup> heteroalleles; six tetrads had a conversion of *URA3*<sup>+</sup> to *HIS3*<sup>+</sup> and one tetrad had a conversion of *HIS3*<sup>+</sup> to *URA3*<sup>+</sup>. The remaining three tetrads segregated 2 *His*<sup>-</sup>*Ura*<sup>+</sup>:1 *His*<sup>+</sup>*Ura*<sup>-</sup>:1 *His*<sup>-</sup>*Ura*<sup>-</sup> spore. One spore in each of these three aberrant tetrads was *His*<sup>-</sup>*Ura*<sup>-</sup> and thus appeared to have neither the *suc2::URA3*<sup>+</sup> nor the *suc2::HIS3*<sup>+</sup> allele. Since two spores in each of these tetrads were *Ura*<sup>+</sup>, it was concluded that the *suc2::HIS3*<sup>+</sup> allele was the one that had been affected during meiosis, giving rise to the *His*<sup>-</sup>*Ura*<sup>-</sup> spore.

**Physical Analysis of DNAs from the Aberrant Tetrads.** There are four possible events that could lead to production

of a His<sup>-</sup>Ura<sup>-</sup> spore in the three aberrant tetrads derived from SJR24. The first event is loss of the *suc2::HIS3<sup>+</sup>* locus by either chromosome nondisjunction or random chromosome breakage. Such an event is easy to detect since it predicts the complete absence of sequences homologous to the *SUC2* locus in the His<sup>-</sup>Ura<sup>-</sup> spores. In Southern blot analyses of genomic DNAs from the aberrant tetrads, however, all spores were found to have *SUC2*-homologous sequences (data not shown).

A second possible explanation for the His<sup>-</sup>Ura<sup>-</sup> spores in the aberrant tetrads is complete loss of the *HIS3* sequences from the *suc2::HIS3<sup>+</sup>* allele. To examine this possibility, we hybridized Southern blots of *EcoRI*-digested genomic DNAs from the aberrant tetrads to *HIS3*-specific sequences. In *EcoRI* digests, the *HIS3* locus is on a 10-kb fragment (24) and the *suc2::HIS3<sup>+</sup>* allele is on a 6-kb fragment (see Fig. 1). If the His<sup>-</sup>Ura<sup>-</sup> spores result from complete loss of the *HIS3* sequences from one of the replicated *suc2::HIS3<sup>+</sup>* alleles during meiosis, then these spores should not have the 6-kb *HIS3*-homologous fragment. Data from the physical analysis of DNAs from a control tetrad (no. 2) and one of the aberrant tetrads (no. 461) are shown in Fig. 3A. As can be seen, the His<sup>-</sup>Ura<sup>-</sup> spore in tetrad no. 461 still has a band of ≈6 kb, which is characteristic of the *suc2::HIS3<sup>+</sup>* allele. The two other aberrant tetrads were identical to no. 461 in a similar analysis (data not shown), and we conclude that the His<sup>-</sup>Ura<sup>-</sup> spores have retained an insertion of *HIS3* information at the *SUC2* locus.

There are two remaining explanations for the occurrence of the three His<sup>-</sup>Ura<sup>-</sup> spores. One possibility is spontaneous mutation of *suc2::HIS3<sup>+</sup>* to *suc2::his3<sup>-</sup>*, and a second possibility is conversion of *suc2::HIS3<sup>+</sup>* to *suc2::his3Δ1*. To distinguish between the mutation and gene conversion hypotheses, we hybridized Southern blots of *BamHI*-di-

gested genomic DNAs from the aberrant tetrads to *HIS3*-specific sequences. The *HIS3<sup>+</sup>* gene inserted into the *SUC2* locus is on a 1.75-kb *BamHI* fragment (see Fig. 1), whereas the *his3Δ1* allele is on a 1.6-kb *BamHI* fragment (20). If the His<sup>-</sup>Ura<sup>-</sup> spores result from a point mutation, one would not expect the size of the *HIS3* insert at the *SUC2* locus to change. DNAs from these spores should therefore have two *HIS3*-homologous bands: a 1.75-kb band corresponding to the presumptive *suc2::his3<sup>-</sup>* mutant allele and a 1.6-kb band corresponding to the *his3Δ1* allele at the *HIS3* locus. If, however, the His<sup>-</sup>Ura<sup>-</sup> phenotype is the result of a gene conversion event, one would expect the His<sup>-</sup>Ura<sup>-</sup> spores to have only the smaller 1.6-kb fragment diagnostic of the *his3Δ1* allele. Fig. 3B shows the results obtained in the analysis of a normal tetrad (no. 2) and one of the aberrant tetrads (no. 461). As expected, all spores have the 1.6-kb *his3Δ1* fragment and the His<sup>+</sup>Ura<sup>-</sup> spores have the larger 1.75-kb *HIS3<sup>+</sup>* fragment; the His<sup>-</sup>Ura<sup>-</sup> spore has only the 1.6-kb *his3Δ1* fragment. Identical results were obtained in analyses of the other two aberrant tetrads (data not shown). We conclude that the His<sup>-</sup>Ura<sup>-</sup> spores are the result of a gene conversion that replaces the *HIS3<sup>+</sup>* information originally inserted into the *SUC2* locus on chromosome IX with the *his3Δ1* allele present on chromosome XV. This event is schematically shown in Fig. 2C. It should be noted that the 6-kb *EcoRI* fragment detected in the His<sup>-</sup>Ura<sup>-</sup> spore shown in Fig. 2A is slightly smaller than the fragment in the His<sup>+</sup>Ura<sup>-</sup> spores. This is exactly the result expected from the conversion of *suc2::HIS3<sup>+</sup>* to *suc2::his3Δ1*.

Meiotic gene conversion is frequently associated with reciprocal recombination of flanking markers (26). To determine whether any of the gene conversions between the *suc2::HIS3<sup>+</sup>* and *his3Δ1* genes were associated with reciprocal recombination, we hybridized Southern blots of *Hpa I*-digested genomic DNAs from the aberrant tetrads to *HIS3*-specific sequences. *Hpa I* cuts asymmetrically in sequences flanking the *HIS3* genes such that a reciprocal recombination event between the genes would produce restriction fragments different from those derived from unrecombined sequences. No evidence of a reciprocal translocation between chromosomes IX and XV was found (data not shown). It should be pointed out, however, that we analyzed only three tetrads and that the relative orientations of the *SUC2* and *HIS3* loci with respect to their centromeres are not known.

**Meiotic Gene Conversion Between *HIS3<sup>+</sup>* and *his3Δ1* at Allelic Positions.** The frequency of meiotic gene conversion between allelic sequences in yeast ranges from 0.5% to 18% (26), and we expected the frequency of conversion between repeated genes on nonhomologous chromosomes to be well below this range. The high frequency (0.5%) of meiotic gene conversion observed between the *HIS3* genes on nonhomologous chromosomes was thus an unexpected finding. One possible explanation for the observed phenomenon is that one or both of the *HIS3* alleles used in the experiment described above are unusually recombinogenic. To examine this possibility, we constructed the control diploid strain SJR33 (*MATa/MATα ura3-52/ura3-52 his3Δ1/HIS3<sup>+</sup> leu2-3, 112/LEU2<sup>+</sup> can1<sup>r</sup>/CAN1<sup>s</sup> suc2::URA3<sup>+</sup>/SUC2<sup>+</sup>*). SJR33 is identical to strain SJR24 except that the *HIS3<sup>+</sup>* gene is located at the normal *HIS3* locus on chromosome XV instead of at the *SUC2* locus on chromosome IX. SJR33 was sporulated and 690 complete tetrads were analyzed. There were 11 conversions at the *HIS3* locus; 8 tetrads segregated 1 His<sup>+</sup>:3 His<sup>-</sup> spores and 3 tetrads segregated 3 His<sup>+</sup>:1 His<sup>-</sup> spore.

The frequencies of gene conversion between the *HIS3* genes in strains SJR24 and SJR33 were compared by determining the Poisson 95% central confidence intervals (27). In strain SJR24, the frequency of gene conversion between the *HIS3* genes on nonhomologous chromosomes was  $4.95 \times 10^{-3}$  (3 events in 606 tetrads), and the 95% central confidence

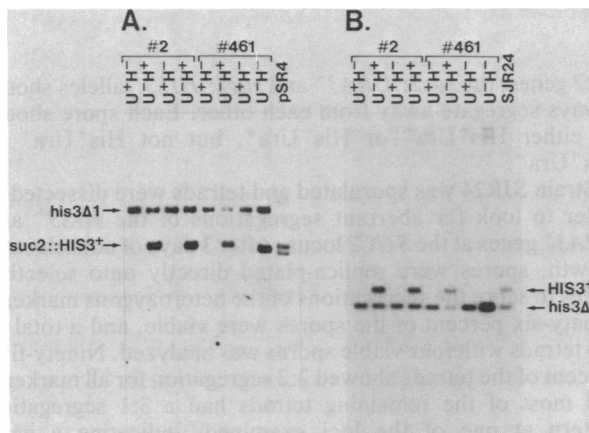


FIG. 3. Southern blot analysis of *HIS3*-homologous sequences in SJR24 tetrads. Yeast DNA for physical analysis was extracted from 5 ml of a stationary culture as described by Sherman *et al.* (19). An aliquot of the DNA was restricted and electrophoresed on an agarose gel in TBE buffer (89 mM Tris/89 mM boric acid/2 mM EDTA) for 16 hr at 2.5 V/cm. DNA fragments were transferred from the gel to a nitrocellulose filter by the method of Southern (25). Filter hybridization was done in Denhardt's solution containing 10% dextran sulfate for 16 hr at 58°C. Filters were hybridized to <sup>32</sup>P-labeled *HIS3* sequences prepared by nick-translating plasmid pSR7 (1.75-kb restriction fragment containing the *HIS3<sup>+</sup>* gene cloned into pBR322). Lanes contain genomic DNAs isolated from SJR24 tetrads no. 2 and no. 461, and are labeled by using the corresponding Ura (U) and His (H) spore phenotypes. (A) DNAs were digested with *EcoRI* and electrophoresed on a 0.6% gel. (B) DNAs were digested with *BamHI* and electrophoresed on a 1% gel. Yeast fragments from the spores that hybridize to the *HIS3* probe are labeled. The large 6-kb fragment from pSR4 corresponds to *suc2::HIS3<sup>+</sup>*; the smaller fragment is 5.5 kb and contains only pBR322-homologous sequences.

interval is  $1.02 \times 10^{-3} < P < 14.5 \times 10^{-3}$ . In strain SJR33, the frequency of gene conversion between the *HIS3*<sup>+</sup> and *his3Δ1* alleles at the *HIS3* locus was  $15.9 \times 10^{-3}$  (11 events in 690 tetrads), and the 95% confidence interval is  $7.96 \times 10^{-3} < P < 28.5 \times 10^{-3}$ . The two confidence intervals clearly overlap, although we note that the interval for strain SJR24 is large. Using the extreme values of the above confidence intervals, we estimate that, at most, the frequency of conversion between allelic *HIS3* genes is 28-fold greater than the conversion frequency between the same *HIS3* genes on nonhomologous chromosomes.

It should be noted that although all conversions between the *HIS3*<sup>+</sup> and *his3Δ1* alleles at the *HIS3* locus can be detected in strain SJR33, one would expect a fraction of the conversions between the *HIS3*<sup>+</sup> gene at the *SUC2* locus and the *his3Δ1* gene at the *HIS3* locus in strain SJR24 to escape detection. Specifically, meiotic conversion of *his3Δ1* to *HIS3*<sup>+</sup> in strain SJR24 would not be detected when the chromosome XV homolog with the *HIS3*<sup>+</sup> gene segregates with the chromosome IX homolog with the *suc2::HIS3*<sup>+</sup> gene. The observed frequency of gene conversion between the *HIS3* repeats in strain SJR24 may thus be an underestimate of the real conversion frequency.

**Mitotic Gene Conversion in SJR24.** The meiotic frequency of gene conversion we observed between the repeated *HIS3* genes on chromosomes IX and XV is at least 3 orders of magnitude higher than that which has been observed mitotically between other yeast genes in different genetic backgrounds (10–14). Since it is important to establish whether the meiotic frequency of such events is indeed higher than the mitotic frequency in the same genetic background, we looked for conversion of *suc2::HIS3*<sup>+</sup> to *suc2::his3Δ1* in the diploid strain SJR24 during mitotic growth. Conversions of this type would result in His<sup>-</sup> segregants of SJR24 that still retain an insertion of *HIS3* information at the *SUC2* locus. Other events that could produce His<sup>-</sup> segregants are allelic mitotic conversion between the mutant *suc2* genes, mitotic crossing-over between the *suc2* genes and their centromeres, and chromosome loss (28). Any of these events would lead to complete loss of the *suc2::HIS3*<sup>+</sup> allele.

Approximately 10,000 colonies from each of 10 independent cultures of SJR24 were screened for a His<sup>-</sup> phenotype by replica-plating onto selective medium. The fractions of His<sup>-</sup> colonies from the 10 cultures were: 0/13,000, 6/11,000, 9/11,000, 1/14,000, 4/9000, 5/9000, 6/15,000, 6/10,000, 6/7000, and 1/9000. Southern blots of *EcoRI*-digested genomic DNAs from nine independent His<sup>-</sup> segregants were hybridized to *HIS3*-specific sequences in order to look for conversions involving the *HIS3* repeats. In all nine isolates examined, only the 10-kb fragment characteristic of the *HIS3* locus was present, indicating complete loss of rather than conversion of the *suc2::HIS3*<sup>+</sup> allele (data not shown).

The meiotic and mitotic frequencies of gene conversion between the *HIS3* genes on nonhomologous chromosomes were compared by determining the Poisson 95% central confidence intervals (27). If we assume that the 35 mitotic His<sup>-</sup> segregants of SJR24 not examined physically were the result of conversion between nonhomologous chromosomes, the maximal mitotic frequency of gene conversion between the *HIS3* genes is  $3.24 \times 10^{-4}$  (35 His<sup>-</sup> segregants in 108,000 colonies) while the meiotic frequency observed was  $4.95 \times 10^{-3}$ . The 95% central confidence intervals for the observed meiotic and estimated mitotic frequencies are  $1.02 \times 10^{-3} < P < 14.5 \times 10^{-3}$  and  $2.26 \times 10^{-4} < P < 4.51 \times 10^{-4}$ , respectively. Since the two intervals do not overlap, it can be concluded that the meiotic frequency of gene conversion between repeated genes on nonhomologous chromosomes is higher than the mitotic frequency.

## DISCUSSION

At a frequency of about 0.5%, we have detected meiotic gene conversion between the *HIS3* genes on chromosomes IX and XV. This frequency of gene conversion was unexpectedly high and, in fact, was not statistically different from the frequency observed in control experiments with the same genes at allelic positions on homologous chromosomes. In agreement with the work of Munz *et al.* (15) with *S. pombe*, we found that the meiotic frequency of conversion between dispersed repeats is higher than the mitotic frequency. Our observed meiotic conversion frequency between the *HIS3* repeats ( $10^{-2}$ – $10^{-3}$ ), however, was much higher than that seen by Munz *et al.* between dispersed tRNA genes ( $10^{-6}$ ); whether this difference is species-specific or is related to the size or sequence of the interacting genes is unclear.

It should be noted that the system used in this study is an alternative to using complementary heteroalleles to select for rare haploid meiotic products that contain both of the relevant genes (29, 30). If one selects for spores having both allelic selectable markers, our system should be able to detect transpositions, chromosome translocations, and gene duplications, in addition to the conversion events reported here. Use of allelic selectable markers also provides a way to select and maintain disomic strains that are otherwise unstable.

About 35% of the meiotic conversion events between allelic sequences in yeast are associated with reciprocal recombination of flanking markers (26). This association has been incorporated into current models of recombination (31, 32), and it is generally assumed that there is an intimate mechanistic relationship between the two processes. In this context, the high rate of gene conversion observed between the *HIS3* genes on nonhomologous chromosomes suggests an interesting paradox. Since the yeast genome contains several classes of dispersed repeated genes, one would expect high rates of meiotic conversion between these repeats to produce numerous chromosome aberrations (translocations, inversions, deletions). Such aberrations, however, are rarely detected in yeast.

There are at least three explanations for the observed absence of chromosome aberrations in yeast. First, it is possible that specific types of gene conversions (for example, events involving dispersed repeats) are resolved without crossing-over (3, 4, 8, 9). To explain the lack of conversion-associated reciprocal exchanges between repeats, it has been suggested that reciprocal recombination might require more extensive sequence homology than a gene conversion event (9, 33, 34), although not all of the fungal data fit this simple hypothesis (35). A second explanation of the paradox is that meiotic gene conversion and reciprocal recombination occur by different mechanisms (35). A mechanistic separation of the two types of recombination has also been suggested to explain data derived from studies of mitotic recombination (36). A third possible explanation is that recombination between naturally occurring repeated yeast genes is suppressed by some unknown mechanism.

In yeast, as in most other eukaryotes, the frequency of genetic recombination between allelic sequences is 3–4 orders of magnitude higher in meiosis than in mitosis (28). One of the most striking cytological features of meiosis in eukaryotes is the precise pairing of homologous chromosomes along their entire lengths and the formation of synaptonemal complexes between the paired homologs (see refs. 37 and 38 for yeast studies). Although the role of the synaptonemal complex is not known, it is generally assumed that the alignment of homologous chromosomes is essential for efficient meiotic recombination (39). In view of the high frequency of meiotic interactions between genes on nonhomologous chromosomes reported here, we suggest either that the complex is not an absolute prerequisite for efficient

meiotic conversion (although it may be required for reciprocal exchange) or that synaptonemal complexes can form between small regions of homology on nonhomologous chromosomes. Although the cytology of yeast is not ideal for observing these complexes, none have been detected between nonhomologous chromosomes (B. Byers, personal communication), even though the yeast genome contains repetitive genes. In addition, experiments in which yeast cells are shifted from sporulation medium to medium permitting a resumption of vegetative growth have indicated that gene conversion can be partly uncoupled from formation of synaptonemal complexes (40). A final possibility is that conversion events between nonhomologous chromosomes involve the interaction of a small diffusible single-stranded DNA fragment (for example, an Okazaki fragment) with a chromosomal locus rather than a direct interaction between two chromosomes.

It is often assumed that gene conversion is a by-product of the requirement for reciprocal recombination in meiosis, but it has also been suggested that the process of gene conversion itself is important in the concerted evolution of dispersed multigene families (41) and in the maintenance of highly polymorphic loci (42). In order for the process of gene conversion to be important evolutionarily, the events must be heritable and therefore, must affect the germ line. We have shown that in the yeast *S. cerevisiae*, conversion between *HIS3* repeats on nonhomologous chromosomes occurs during meiosis, and is, in fact, more frequent than the analogous mitotic conversion. Evidence for gene conversion has also been obtained in mammalian systems (43–45), and Loh and Baltimore (46) have suggested that some of these conversion events occur in meiosis. The high rate of meiotic conversion between repeated genes reported here, therefore, is likely to be a general phenomenon rather than one restricted to fungal systems.

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