Meiotic Recombination in Yeast: Alteration by Multiple Heterozygosities

RHONA H. BORTS AND JAMES E. HABER*

Although meiotic gene conversion has long been known to be accompanied by crossing-over, a direct test of the converse has not been possible. An experiment was designed to determine whether crossing-over is accompanied by gene conversion in *Saccharomyces cerevisiae*. Nine restriction site heterologies were introduced into a 9-kilobase chromosomal interval that exhibits 22 percent crossing-over. Of all the exchange events that occurred, at least 59 percent of meiotic crossovers are accompanied by gene conversion of one or more of the restriction site heterologies. The average gene conversion tract length was 1.5 kilobases. An unexpected result was that the introduction of as few as seven heterozygosities significantly altered the outcome of recombination events, reducing the frequency of crossovers by 50 percent and increasing the number of exceptional tetrads. This alteration results from a second recombination event induced by repair of heteroduplex DNA containing multiple mismatched base pairs.

MEIOTIC GENE CONVERSION, THE NONRECIPROCAL transfer of genetic information, is accompanied by reciprocal recombination. Between 30 and 70 percent of gene conversion events are associated with a crossover in flanking genetic intervals (*1*). Several molecular models have been proposed to explain the known properties of gene conversion (polarity, parity, coconversion, postmeiotic segregation, and the association of crossovers with conversions). In both the Meselson-Radding (*2*) modification of the Holliday model (*3*), and the double-strand break repair model of Szostak *et al.* (*4*), gene conversion whether with or without exchange is initiated by the same mechanism. However, the data obtained from studies of a number of organisms concerning the length of conversion tracts and position of crossovers relative to the conversion segment have led to suggestions that there may be two or more mechanisms (*5, 6*). For example, it is possible that there are different recombinational pathways, such as those that yield reciprocal exchanges that are not associated with a gene conversion event and those that are (*5*). Although Hurst *et al.* (*7*) have calculated that the amount of crossing-over associated with gene conversion is sufficient to account for the genetic map distances in *Saccharomyces cerevisiae*, there has been no direct test of the hypothesis that most meiotic recombination events arise from the same mechanism that yields gene conversions.

We have created a 9-kilobase (kb) interval within which 22 percent of all tetrads exhibit crossing-over (*8*). Between the flanking markers we have placed a number of heterozygous restriction site mutations so that we could monitor gene conversion in this interval. We find that a minimum of 59 percent of all reciprocal exchanges are accompanied by a gene conversion event, with an average conversion tract length of approximately 1.5 kb. Unexpectedly, however, the introduction of multiple heterozygosities significantly alters the kinds of recombination events that are recovered. We believe this perturbation reflects a second, repair-induced recombination event.

The introduction of multiple heterozygosity decreases reciprocal exchange. A multiply marked interval was created by the integration of mutated pBR322 sequences at the mating-type locus (*MAT*) of yeast (Fig. 1A). Tetrads in which reciprocal crossing-over within the *MAT-URA3*-pBR322-*MAT* interval has occurred can be recognized because two of the four meiotic products give rise to haploid nonmating spore colonies of genotypes *MAT***a**-*URA3*-pBR322-*MAT*α and its reciprocal, *MAT*α-*URA3*-pBR322-*MAT***a**, because of the codominant expression of *MAT***a** and *MAT*α (Fig. 1B). The other two spores give rise to **a**-mating and α-mating colonies with parental configurations of the *MAT* loci. When there is no heterozygosity in this interval, approximately 22 percent of all tetrads contain such an exchange event (Table 1). The introduction of the nine heterozygosities shown in Fig. 1 reduces the frequency of crossing-over approximately twofold (Table 1). This reduction in reciprocal recombination is not caused by a particular mutation or the set of mutations within this interval, because diploids homozygous for one parental configuration or the other (Table 1, diploids RHB556, 562, 589, and 597) are indistinguishable from a diploid with wild-type pBR322 sequences (Table 1, RHB566). As is discussed below, there is no net loss of recombination events in the interval; instead, there is a change in the way that approximately half of the events are resolved.

Reciprocal recombination is accompanied by gene conversion. Restriction enzyme digestion of DNA from segregants of a diploid with heterozygous restriction sites yields different sized fragments for all parental and recombinant structures. We can thus determine in which region reciprocal exchange has occurred and if any restriction sites have been converted. A representative exchange (Fig. 1) is shown as the formation of heteroduplex DNA, and this is followed by mismatch repair (and gene conversion) at the Pst I, Nde I, and Pvu II sites. The data obtained by Southern (*9*) blotting are shown for five of the nine sites (Fig. 1C). In this tetrad, spores C and D are recombinant. The crossover point maps between the centromere-proximal Xho I site and the Sma I site. The region including both the Pvu II and the Pst I sites was converted to their mutant alleles in the *MAT***a**-*URA3*-pBR322-*MAT*α recombinant.

The authors are at the Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, MA 02254.

*To whom correspondence should be addressed.

Of the events observed in 49 crossover tetrads from RHB536 (Fig. 2), 41 percent (20 of 49) were crossovers with no detected conversion event. The remaining 59 percent (29 of 49) converted one or more restriction sites. Models in which gene conversion results from mismatch repair of heteroduplex DNA (*2, 3*) predict that no more than 50 percent gene conversion would ever be found because heteroduplex DNA can either be restored to the parental allele or converted to the nonparental allele with equal frequency. Conversely, since the double-strand break model (*4*) postulates that every exchange is initiated by a double-strand gap that must be repaired from the unbroken homolog, it predicts that if it were possible to assay conversion at every site between the flanking markers, every event would contain evidence of a gene conversion. Although our data are in good agreement with a heteroduplex model, we cannot assay every site and therefore cannot rule out the double-strand break model. Other data, discussed below, are not consistent with a double-strand break initiating meiotic recombination.

In most conversion events (26 of 29) the crossover point is not separated from the conversion event and therefore cannot be mapped to one side or the other of the conversion tract. In three events, including the one diagrammed in Fig. 1, the exchange event is separated from the conversion by unconverted (or restored) sites. We also found one event in which there were two separate conversion tracts. We cannot determine whether these were two independent events or whether a long region of heteroduplex was corrected so that the intervening markers were restored. We see only one event that appears to be best explained by the formation of

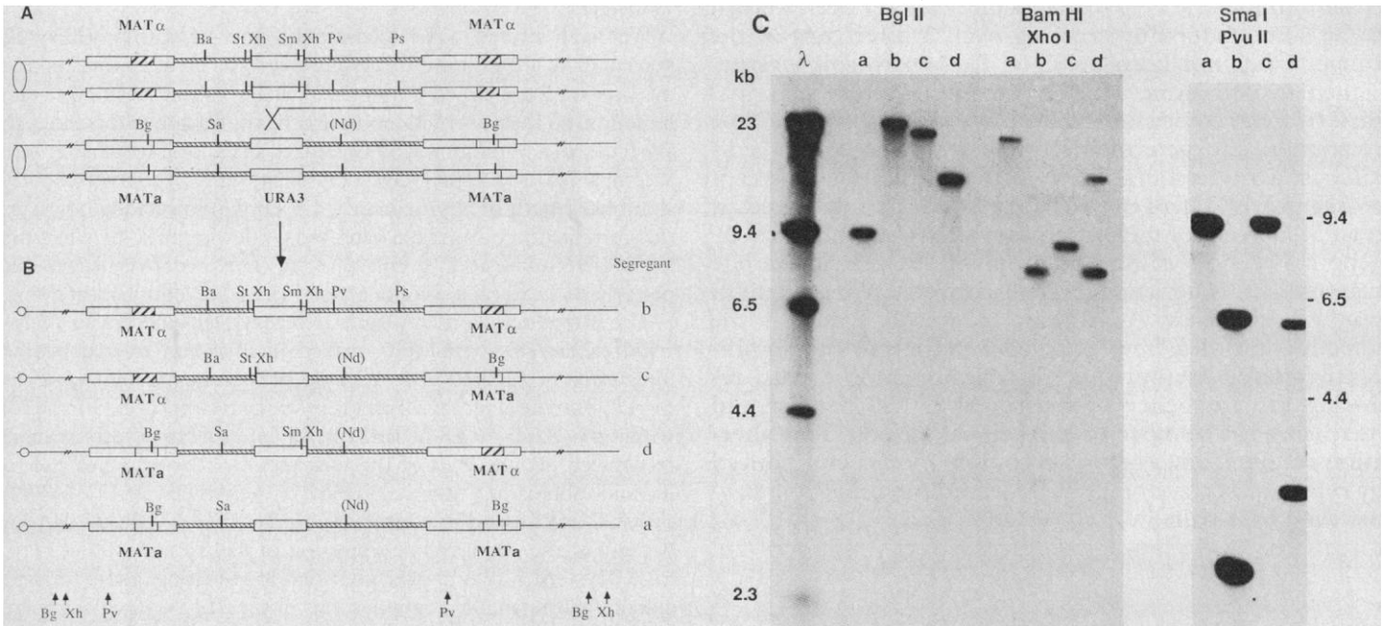

Fig. 1. Parental configuration of the *MAT-URA3*-pBR322-*MAT* region and the restriction site polymorphisms. (**A**) The structure of the *MAT***a**-*URA3*-pBR322-*MAT***a** and the *MAT*α-*URA3*-pBR322-*MAT*α parents. The restriction sites indicated are those present in each parent. Centromeres (circles) are to the left of the *MAT* loci. The initiation of an exchange event, including the asymmetric formation of heteroduplex DNA, is indicated. (**B**) The recombination event in tetrad RHB536-186. A reciprocal crossover within the interval defined by the *MAT***a** Bgl II (Bg) sites (*MAT***a** contains a Bgl II site not found in *MAT*α) produces a tetrad containing two parental, **a**- or α-mating spores (designated a and b) and two haploid, nonmating spores (c and d). From the pattern of the restriction site markers for each of the four spores in this tetrad (C) the exchange event appears to have occurred between the centromere proximal Xho I site and the Sma I site. In addition, the Pvu II and Pst I sites have been converted to the mutant alleles. Restriction endonuclease sites common to both chromosomes and important for mapping the sites within the *MAT-URA3*-pBR322-*MAT* interval are indicated below; also indicated are a single Pvu II site in each *MAT* locus, and Bgl II and Xho I sites outside the 9-kb interval. Events at the Nde I site (in parentheses) were not analyzed. (**C**). Southern analysis of recombinant tetrad RHB536-186. DNA from all four spores of this tetrad were digested with Bgl II, Bam HI + Xho I, and Sma I + Pvu II. The restriction endonuclease digests were separated by electrophoresis on a 0.5 percent agarose tris-borate-EDTA gel, transferred to a nitrocellulose filter (*9*), and probed with ^{32}P-labeled pBR322. (Lane a) The DNA is from the parental *MAT***a** spore colony and contains a 9-kb restriction fragment homolgous to the pBR322 probe. (Lane b) DNA from a parental *MAT*α colony that lacks the *MAT***a** Bgl II sites and yields a 31-kb fragment. (Lanes c and d) The recombinant DNA from the Bgl II sites yields the 26-kb and 14-kb Bgl II fragments expected for a *MAT*α-*URA3*-pBR322-*MAT***a** and *MAT***a**-*URA3*-pBR322-*MAT*α, respectively. Segregant a lacks both the Bam HI and Xho I sites within the interval and thus yields a 24-kb restriction fragment. DNA from segregant b is cleaved into a strongly hybridizing 8-kb band and two less strongly hybridizing 1-kb and 15-kb bands. With segregant c, the strongly hybridizing band is 9 kb, expected if the distal Xho I site is absent; the two weakly hybridizing bands are unchanged. Segregant d contains neither the Bam HI site nor the proximal Xho I site and therefore yields a 16-kb fragment as well as an 8-kb fragment. Similar analysis of Sma I + Pvu II digests shows that both the parental segregant a and the recombinant segregant c contain a single, 9-kb restriction fragment derived from cutting the two Pvu II sites in the duplicated *MAT* regions. Segregant b contains three smaller bands reflecting the presence of both a Sma I and a Pvu II site within *URA3* and pBR322. DNA from segregant d gives rise to two bands expected if the Sma I site in *URA3* is present but the Pvu II site in pBR322 is absent. Similar analyses were carried out for Sty I + Pst I, and Sal I to determine the presence or absence of these restriction sites within the 9-kb interval.

symmetric heteroduplex, such that repair of the symmetrical heteroduplex to the nonparental allele on both strands results in an apparent triple exchange.

Absence of preferred sites for crossing-over or gene conversion. The number of times that each site participated in a gene conversion was calculated for the 29 cases described above. All sites appear equally likely to be involved in a conversion event; among the 49 tetrads, the probability that any one site is converted is 16.8 ± 4.5 percent. For those events where the site of exchange could be unambiguously determined, 15 of 23 exchanges were located in the 3-kb interval between the Sty I and Pvu II sites; however, the location of crossovers is statistically indistinguishable from a distribution proportional to the distance between restriction site markers.

We have found no evidence for disparity among the single site conversions of the restriction site mutations. Of 11 conversions, 5 were to the small deletion; 6 of 11 resulted in an insertion. These data are supported by a similar parity in gene conversions of similar small insertions or deletions at restriction sites in *leu2* (*10*) and *his4* (*11*).

We previously reported that, when *URA3* was inserted into wild-type pBR322 sequences, the frequency of crossing-over between flanking *MAT* sequences was approximately three times greater than that observed when *URA3* was absent (*8*). Diploids constructed in the Y55 strain background used in our experiments do not show this effect. The *MAT*a-pBR322-*MAT*a and *MAT*α-pBR322-*MAT*α derivatives (referred to as URA^0) were constructed and mated to each other to make diploids RHB569 and RHB570. We also constructed URA^0 derivatives that were homozygous for the pBR322 mutations (RHB589 and RHB597). The frequency of reciprocal recombination in these strains, with seven heterologies, is not significantly different from that of the *MAT-URA3*-pBR322-*MAT* diploids (Table 1).

The average conversion tract is 1.5 kb. An estimate for the minimum average length of conversion tracts was calculated by assigning to simple crossovers a conversion tract length of zero, to single-site conversions a value of 0.005 kb, and to coconversions the length between the outermost involved sites. For the 49 events (Fig. 2), we calculate a minimum average conversion tract length of 0.4 kb (standard deviation ± 0.8 kb). A maximum conversion tract length of 2.6 ± 1.9 kb was estimated from the distance between the two nearest unconverted flanking sites. The average midpoint estimate was taken to be halfway between the minimum and maximum value for each event; this yields an average conversion tract length of 1.5 ± 1.3 kb.

The introduction of multiple heterozygosities into a region increases the frequency of nonreciprocal recombination. As indicated above, the presence of multiple heterozygosities reduced the frequency of reciprocal exchanges approximately twofold. Concomitantly, there was an increase in nonreciprocal recombination events (Table 2). An increased number of tetrads contained three mating spores and one nonmating spore. Most of these tetrads involved conversions of one *MAT* locus to the opposite mating type, to yield a haploid nonmating segregant (class A, Table 2). Wild-type and homoallelic diploids undergo this type of event approximately 1 percent of the time, whereas multiply heterozygous

Table 1. Effect of heterozygosity on reciprocal crossing-over. All strains are derived from strains backcrossed a minimum of three times to strain Y55. Diploids RHB619, 566, 569, and 536 are completely isogenic.

RHB strain	Structure*	Number heterozygosities	Frequency of reciprocal exchange†
	Homozygous diploids		
556	a-URA-x-a α-URA-x-α	0	85/400 (21.5)
562	a-URA-x-a α-URA-y-α	0	97/410 (23.6)
619	a-URA-x-a α-URA-x-α	0	63/249 (25.9)
589	a-x-a α-x-α	0	54/311 (17.4)
597	a-y-a α-y-α	0	67/325 (20.6)
	Heterozygous diploids		
566‡	a-URA-w-a α-URA-w-α	3	91/409 (22.2)
536	α-URA-y-α a-URA-x-a	9	47/435 (10.8)
569	a-x-a α-y-α	7	39/401 (9.7)
570	a-x-a α-y-α	7	21/198 (10.6)
604	a-x-a α-y-α	7	48/329 (14.6)

*x refers to the Bam HI$^-$, Sty I$^-$, Sma I$^-$, Pvu II$^-$, and Pst I$^-$ pattern of markers, y refers to the Sal I$^-$, Xho I$^+$, Nde I$^-$ restriction site pattern (see Fig. 1), and w refers to wild-type pBR322 sequences. †Number of tetrads containing two reciprocal haploid nonmating spores out of the total number of four spored asci analyzed. The percentage is given in parenthesis. ‡In strain RHB566 the pBR322 sequences are wild type. The *URA3* genes are nonhomologous at the Sma I site and at the junction of the *URA3* insert into the Ava I site of pBR322.

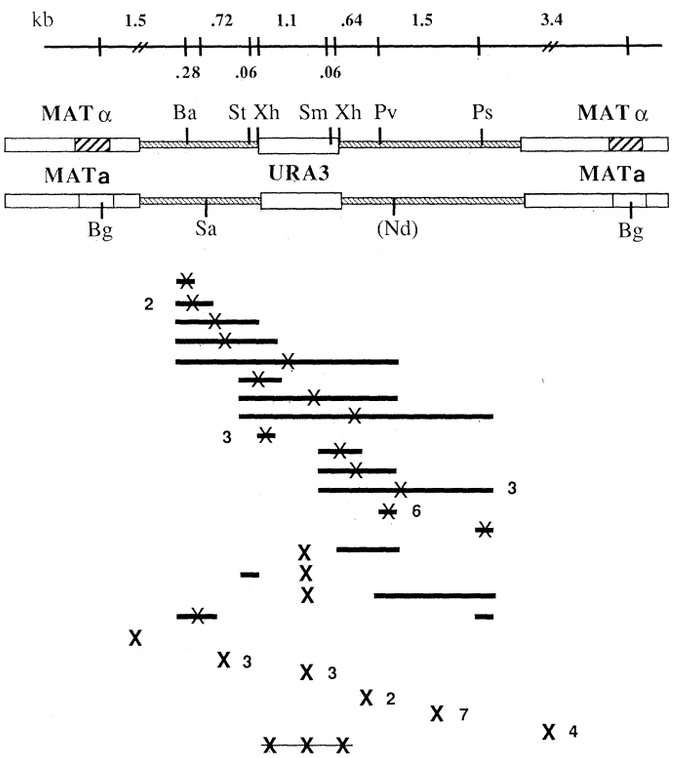

Fig. 2. Map of crossover points and gene conversions. Forty-nine pairs of reciprocal recombinants from RHB536, whose structure is shown, were analyzed (Fig. 1). The locations of crossovers without conversion (X), single site conversions and coconversions (—X—) and crossovers separated from the conversion (X —) are indicated beneath a map of the interval. Events that occurred more than once are indicated by the adjacent number. The event shown at the bottom can either be explained by a triple crossover in the interval indicated, or it may have resulted from the independent mismatch correction of heterozygous sites in a region containing symmetrical heteroduplex DNA. The distances between the sites are given in kilobases.

diploids have *MAT* conversions 5 percent of the time.

The *MAT* conversion events were further analyzed to determine the extent of the conversion tract and to determine whether a reciprocal exchange had occurred within the *MAT*-pBR322-*URA*-*MAT* interval. The nonmating colony and the two mating colonies bearing the chromosome with which it could have interacted were analyzed by Southern blots for the presence or absence of restriction sites (Fig. 3). Of the 24 events analyzed, 67 percent (16 of 24) had a conversion tract that extended from the converted *MAT* locus to at least the first pBR322 marker. Five of the 24 *MAT* conversions apparently also involved exchanges with the chromosome of the opposite mating type, because one of the two mating colonies with which it could have interacted contained nonparental restriction sites (Fig. 3). The frequency of events involving only the *MAT* locus and no pBR322 sequences (1.2 percent of all tetrads) is the same as that found in homozygous diploids (1.0 percent) and may represent normal *MAT* conversion events (*12*). A minimum estimate of the conversion tract length associated with *MAT* conversions was estimated on the assumption that the tract did not extend outside the interval, that is, beyond the converted *MAT* locus. This minimum value of 2.0 ± 1.7 kb for the 24 *MAT* conversions is substantially larger than the 0.4 ± 0.8 kb minimum tract length calculated above for crossover tetrads ($P < 0.001$, based on a standard normal Z test).

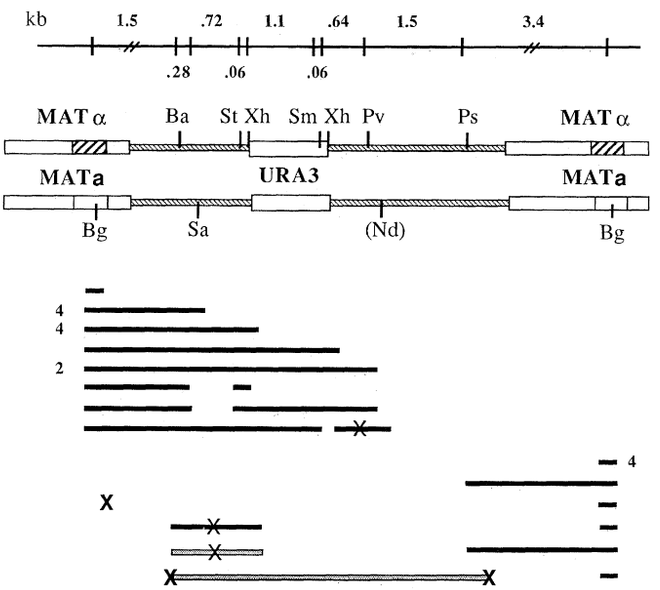

Fig. 3. Twenty-four tetrads from RHB536 containing a single nonmating segregant were also analyzed (Fig. 1). The single nonmaters and the two segregants that might have served as donors for the conversion event were analyzed. Single-site conversions and coconversions are indicated as above. Conversions that occurred on the strand opposite from the *MAT* conversion are indicated by a stippled bar. Unlike the events in Fig. 2, only five events (X) were accompanied by a crossover in the *MAT*-*MAT* interval.

A second class of events, in which one spore had become both Ura$^-$ and pBR322$^-$ (from initially Ura$^+$ diploids), or pBR322$^-$ (in URA^0 diploids), also occurred at a significantly increased frequency. In the diploids heterozygous for the pBR322 mutations, the frequency of such tetrads was 5.7 percent (Table 2, classes B through I), as compared to 1.0 percent in diploids that were homozygous for either set of restriction site markers or homozygous for wild-type pBR322 sequences. Southern blot analysis of DNA made from the four segregants of tetrads containing these events allowed us to determine the nature of the event leading to the Ura$^-$ pBR322$^-$ structure. An intrachromatid crossover between the two *MAT* loci (Table 2, class B) or an unequal sister chromatid exchange (Table 2, class D) give tetrads with four mating spores, one of which lacks pBR322 sequences. Digestion of DNA with an enzyme that cuts once within the pBR322 sequences from the spore with the same mating type as the pBR322$^-$ spore can distinguish these two events. The product of an unequal sister chromatid exchange (*MAT*-pBR322-*MAT*-pBR322-*MAT*) can be identified by an extra restriction fragment homologous to pBR322, not found in a simple *MAT*-pBR322-*MAT*. Similarly, the class E tetrad appears to have arisen from an unequal interchromosomal exchange event in which one of the interacting *MAT* loci had also undergone gene conversion.

One other type of tetrad (class C) was much more prevalent in tetrads derived from the multiply heterozygous crosses. These appear to contain the products of both an intrachromosomal exchange (yielding a pBR322$^-$ spore) and a segregant in which one of the flanking *MAT* loci had undergone gene conversion. The frequency of these tetrads (11 of 633, 1.8 percent) is substantially

Table 2. Analysis of aberrant tetrad types. A representative tetrad from each class is illustrated. The abbreviated structure **a**-α represents a *MAT***a**-pBR322-*MAT*α structure. Other configurations of the mating-type loci are possible in all classes. For example, a tetrad with α-**a**, **a**-**a**, **a**-**a**, and α-α spores also belongs in class A. Similarly, a tetrad with α, α-α, **a**-**a**, **a**-**a** segregants also belongs in class B. Diploids RHB536, 562, 556, and 566 all contain *URA3* inserted in the pBR322 interval, while diploid RHB570 does not.

RHB strains	Number of tetrads in class									Ratio of number of nonreciprocal events to number of tetrads
	A	B	C	D	E	F	G	H	I	
	a-α **a**-**a** α-α α-α	**a** **a**-**a** α-α α-α	**a**-α **a** **a**-**a** α-α	**a**-**a**-**a** **a** α-α α-α	**a**-α-α α α-α **a**-**a**	α-α-**a** α **a**-α **a**-**a**	**a**-α α-**a** **a** α-α	**a** **a** α-α α-α	Other	
					Nine heterologies					
536*	24	9	8	2	0	2	2	1	0	48/435 (11%)
					Seven heterologies					
570†	13	6	3	2	1	2	0	0	0	27/198 (14%)
					Three heterologies					
566*	3	0	1	1	2	0	1	1	1	10/409 (2.4%)
					Zero heterologies					
562* 556	10	1	1	0	0	1	0	0	2	15/819 (1.8%)

*Spore colonies that had lost the *URA3* pBR322 sequences were identified by their Ura3$^-$ phenotype and were confirmed by Southern blot analysis. †All 198 tetrads were screened for loss of pBR322 sequences by yeast colony hybridization (*20*) with α-^{32}P-labeled pBR322 as a probe. In a double-blind reconstruction experiment of the colony hybridization technique, all pBR322-containing colonies were identified. All negatives (and putative negatives) were retested for loss of pBR322 sequences by dot blots or Southern analysis of purified DNA.

higher than that expected for coincidental, but independent, *MAT* conversion and intrachromosomal exchange (0.3 percent).

The tetrads in classes G and H are more complex and appear to have involved at least three chromatids. Class G tetrads exhibited a reciprocal exchange (to yield a-α and α-a segregants) and an intrachromatid crossover; the frequency of these tetrads was consistent with the occurrence of two independent events. The four class F tetrads had one nonmating spore with the structure of a *MAT* conversion and two other segregants with structures consistent with their having been an unequal interchromosomal crossover between them (that is, a single *MAT* locus in one segregant and a triplication of the *MAT* locus in another). The frequency of these events (0.6 percent) is too high to have resulted from independent *MAT* conversion and unequal interchromosomal exchange events (0.09 percent).

The members of class I ("other") were found only in the homozygotes and also represent three strand events. Two of these events involved a net gain of plasmid sequences. In one member of this class, the two nonmaters had the structure of *MAT* conversions and may have been the product of coincidental class A and class C events. The last class of tetrads found contained two Ura$^-$ spores of the same mating type (class H). We cannot determine whether these arose by a mitotic unequal exchange, or whether they arose by two independent meiotic intrachromatid crossovers between the flanking *MAT* loci.

Effect of multiple heterozygosities on the spectrum of conversion events. The location and extent of gene conversion and reciprocal exchange were analyzed in strain RHB566, in which only the two Xho I sites and the Sma I site were heterozygous. The overall frequency of crossing-over in this diploid was twice as high as in the multiply heterozygous diploid (RHB536) and was equal to that of the completely homozygous diploids (Table 1). Of the 43 events analyzed, 20 (46.5 percent) contained a gene conversion or crossover (or both) in the region defined by the *URA3* gene (Fig. 4A). When the 49 reciprocal exchange events in the multiply heterozygous diploid, RHB536 (Fig. 2), are analyzed with respect only to what occurred at the Xho I and Sma I sites, a similar distribution of gene conversion and exchange is seen (Fig. 4B). Thus, among the reduced number of tetrads with a reciprocal exchange yielding two nonmaters, the presence of multiple heterozygosities does not seem to alter either the length of conversion tracts or the distribution of the crossovers themselves.

Multiple heterologies appear to induce a second recombination event. We have introduced a set of nine restriction site mutations into a small, well-defined interval, only to discover that the presence of these heterologies perturbs the meiotic recombination events we expected to monitor. The frequency of reciprocal crossover events fell from approximately 22 percent when the region was homozygous to 10 to 15 percent when heterozygous for the nine restriction site ablations. The loss of exchange events was accompanied by an approximately equal increase in other classes of events, especially gene conversion of one of the flanking *MAT* markers or the apparent excision of the sequences between the flanking *MAT* genes. We propose that the loss of simple reciprocal recombinants and the increase in the other classes stems from repair of heteroduplex DNA generated during reciprocal recombination in this interval. We suggest that (i) the repair event creates a double-strand break, or possibly an extensive length of single-stranded DNA, that is a very active substrate for recombination, and (ii) the aberrant structures result from a second, repair-induced recombination event initiated by this substrate.

In essence, the sequence of events outlined embodies the most attractive features of both the single-strand initiation (*2*) and the double-strand break repair (*4*) models. Hastings (*13*) has suggested that mismatch repair events, especially multiple, independent events within a region containing heteroduplex, might generate double-strand breaks or gaps. Such breaks could be created by converging excision-repair enzymes operating on opposite strands of the same heteroduplex (Fig. 5B). These breaks would subsequently be repaired by the gap repair mechanism suggested by Szostak *et al.* (*4*). Repair of these double-strand breaks could involve either sister chromatids or nonsisters, and could thus result in crossing-over of strands not involved in the primary event that generated the heteroduplex. In this fashion we can account for a number of the aberrant tetrad types that increase when the region contains a large number of heterologies.

The large increase in *MAT* conversions (class A) can be explained (Fig. 5D). A gap created during repair of heteroduplex DNA would be expanded to include the *MAT* region that was not initially included in heteroduplex DNA. This may be the reason why these *MAT* conversion tracts are significantly longer, on average, than those found for tetrads with only a reciprocal exchange within the interval (Fig. 2). An alternative interpretation might be that *MAT* regions are frequently included in heteroduplex DNA, even in diploids homozygous for markers in the interval, but that the *MAT* loci are not converted unless adjacent heterologous sites are also converted. If this were the case, in diploids homozygous for the markers, one would then expect to see postmeiotic segregation (PMS) for these *MAT* regions. Such PMS events would appear as sectored colonies in which one-half was nonmating (because it had an a-α or α-a structure) and the other half-sector exhibited normal a- or α-mating. No such PMS events were detected among 236 tetrads of RHB566 and related strains homozygous for the markers in pBR322. Therefore increased *MAT* conversions may depend on repair-induced events that extend into the flanking region.

The other large class of unusual events involves apparent intrachromatid exchanges between the flanking *MAT* markers, thereby excising the pBR322 sequences. In half of these instances, another chromatid displayed an exchange between the flanking markers.

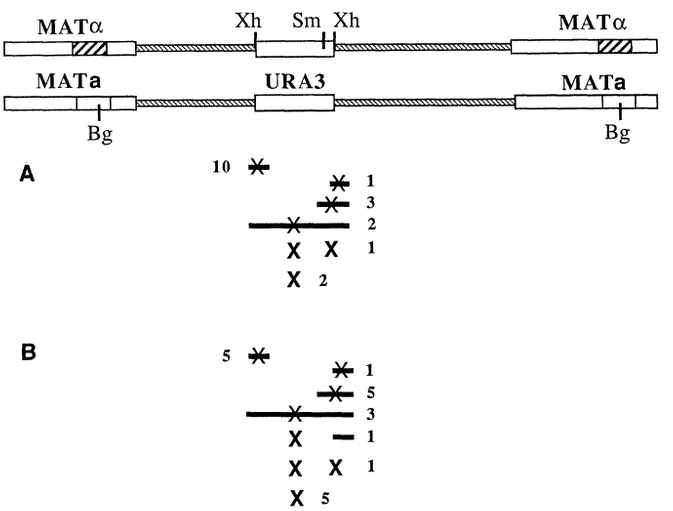

Fig. 4. Comparison of the distribution of crossovers and gene conversions in diploids RHB536 and RHB566. (**A**) Forty-three pairs of reciprocal recombinants from diploid RHB566 were analyzed for the presence or absence of the two Xho I sites and the Sma I site as described (Fig. 1). Diploid RHB566 differs from RHB536 (Fig. 2) in that it is heteroallelic only at the two Xho I and the Sma I sites. All other sites are homozygous wild type. The location and number of gene conversion and reciprocal exchanges is indicated beneath a map of the interval. The symbols used are as in Fig. 2. (**B**) The 49 events from RHB536 (Fig. 2) were analyzed only for gene conversions that encompassed the two Xho I and Sma I sites. When the 21 tetrads that had conversions of one or more of these three sites were analyzed in this fashion, the distribution of events shown is obtained.

These tetrads may have arisen by a novel form of double-strand break repair in which recombination occurs between the two flanking regions of homology (in this case the *MAT* loci). Thus, creation of a double-strand break during repair of heteroduplex DNA formed by a reciprocal exchange event may also yield a tetrad with one reciprocally recombined segregant and one "looped out" (Ura^-, $pBR322^-$) derivative (Fig. 5E). In other experiments we have shown that recombination between homologous regions flanking a double-strand break occurs very frequently in mitotic yeast cells when the *HO* endonuclease is used to generate a double-strand break in a chromosomal region flanked by *URA3* genes (*14*).

We have been able to demonstrate multiple, sequential recombination events primarily because of the presence of the nontandem duplication of the *MAT* loci that permits repair by intrachromosomal recombination. In the absence of these flanking regions, most multiple recombination events would be undetectable in standard genetic crosses involving only a few heterozygous sites. However, several types of events seen in previous studies of gene conversion appear to reflect a sequence of a recombination event followed by a second, repair-induced recombination.

The possibility that repair-induced recombination may also be accompanied by crossing-over implies that some events that initially occur without crossing-over could subsequently be repaired to include an exchange of flanking markers. This second round of recombination can explain such long-standing problems as how a region of heteroduplex can have an exchange event close to the site of initiation of heteroduplex DNA formation rather than at the termination of the heteroduplex region (*15, 16*). Similarly, two sequential recombination events may explain cases in which crossovers have been separated from the original conversion tract.

A second round of recombination resulting from heteroduplex DNA repair also accounts for the recovery of tetrads in which three chromatids were involved in what appeared to be a single event (classes F, G, and I). Evidence for three-strand events has also been reported in studies of gene conversion at several well-studied loci in *Saccharomyces* (*15–17*). We suggest that three-strand events associated with gene conversion reflect the action of a second repair-induced recombination event in the absence of flanking regions of homology.

Formation and repair of heteroduplex DNA. In addition to revealing a second round of (repair-induced) recombination in meiosis, our data also help clarify several aspects of the mechanism of meiotic recombination in *Saccharomyces*.

1) The observation that the presence of as few as seven heterologies within the 9-kb region exerts such a great effect leads us to suggest that meiotic recombination often involves the formation of extended regions of heteroduplex DNA. This would seem to exclude double-strand breaks as a model for initiating meiotic recombination (*4*); one of the intrinsic features of the double-strand break repair model is that only short regions of heteroduplex DNA are generated adjacent to a repaired gap. We would not expect multiple heterologies to affect either the creation of double-strand breaks to initiate meiotic crossing-over or the resolution of gap repair events.

2) We observe a strong correlation between reciprocal exchange and gene conversion. In previous studies, where gene conversion events were selected, approximately half of these events were associated with crossing-over (*1, 15, 17*). Those results did not preclude the possibility that many reciprocal exchange events proceeded by a pathway that did not involve an associated gene conversion (*5*). Our data show that 59 percent of reciprocal crossovers were accompanied by gene conversion. Similar results have been obtained by Symington and Petes (*18*) in a study of meiotic recombination and gene conversion in the chromosomal interval between *LEU2* and the centromere of chromosome III. This is a minimum estimate, as some conversion events will have been too short to have crossed over a marker. Thus, it is possible that all or most crossovers are associated with a conversion event. Alternatively, our data are consistent with the hypothesis that, while all crossovers are associated with the formation of heteroduplex DNA, some heteroduplex DNA remains undetected because it is restored to the parental genotype.

Most of the gene conversion events that we have analyzed are

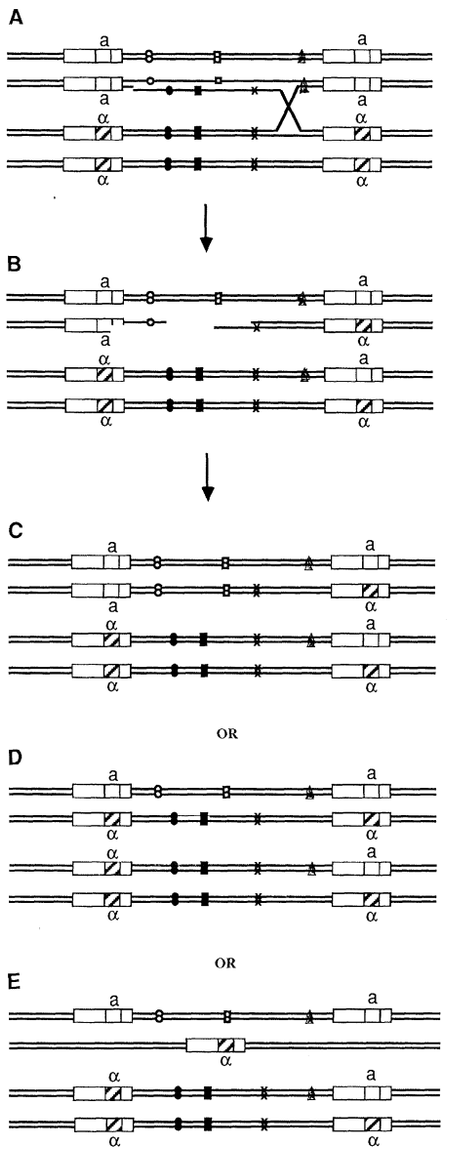

Fig. 5. Heteroduplex DNA repair-induced recombination in an interval that has already undergone an exchange event. As illustrated, the primary reciprocal recombination event involved the formation of a long region of heteroduplex DNA (**A**). The convergence of two independent repair events leads to the formation of a double-strand-break or gap (**B**) as suggested by Hastings (*13*). This gap may be enlarged. The broken chromatid can be repaired (*4*) with either a sister (**C**) or nonsister (**D**) chromatid as a template. If a sister chromatid was used as a template for repair of the double-strand break (C), sites initially included in heteroduplex DNA might be restored to their parental configuration. If a nonsister chromatid was involved in double-strand gap repair (D), a tetrad of class A can be recovered (Table 2). Alternatively, the broken chromatid can be repaired by an intrachromosomal recombination event involving flanking regions of homology (in this case, the *MAT* regions), to yield one segregant that has lost the plasmid sequences (**E**). These novel classes of tetrads (classes B and C in Table 2) are found frequently in diploids carrying multiple heterozygosities in the *MAT-URA3*-pBR333-*MAT* interval.

continuous, rather than interrupted. Of 12 instances in which the conversion event spanned three or more markers (Fig. 2A), only three appear to contain conversion tracts separated by an unconverted or restored marker. The relative absence of independently repaired sites, separated by more than 1 kb, can be explained if the initiation of two or more independent corrections of heteroduplex DNA frequently results in double-strand breaks and the subsequent second round of recombination. Symington and Petes (*18*) have found only three examples of an interrupted conversion tract in 33 events in the *LEU2-CEN3* region.

Kolodkin *et al.* (*19*) have suggested that double-strand breaks arising during meiosis might be preferentially repaired with an intact sister chromatid as a template. We cannot measure how often sister chromatids are used to repair double-strand breaks, but we do point out that repair events apparently with nonsister chromatids (class A, Table 2) and homologous sequences on the same chromatid (classes B and C, Table 2) appear to occur with equal frequency. Thus nonsister chromatids contribute significantly to the repair of heteroduplex DNA.

The spectrum of conversion events around the *URA3* segment found in RHB566, a diploid that bears three sequence heterologies, was nearly identical to that found for reciprocal recombinants carrying nine heterologies. This may be because conversion tracts that span more than three heterologies are potential substrates for repair-induced recombination, and therefore may not be recovered in the class of reciprocal crossovers. Thus, the events remaining in the class of reciprocal recombinants are similar to those found when there are fewer heterologies. This interpretation has the further implication that we may be underestimating the average conversion tract length in yeast.

Finally, we gain some insight into the extent of mismatched heteroduplex DNA that must be formed in order to generate a repair-induced recombination. In one series of experiments we used diploids homozygous for all sites except the Xho I and Sma I sites flanking and within the *URA3* gene (RHB566). These three heterologies did not result in a loss of reciprocal exchanges or an increase in aberrant events. Possibly, repair-induced recombination might not occur unless there are mismatches more than 1.2 kb apart or unless more than three heterologies are included in heteroduplex DNA. Alternatively, it seems quite likely that the reduction in exchanges in the interval and the increases in both *MAT* conversions and intrachromatid exchanges in the multiply heterozygous diploid might depend primarily on the proximity of the repair-induced lesion to one of the flanking homologous sequences. Thus, detection of these aberrant events may depend on heterologies closer to these regions. Mismatch repair-induced recombination events further from the flanking homologous regions would be repaired with a sister or nonsister chromatid as a template and would only be detected when gene conversion and its associated exchange appeared to involve three chromatids.

REFERENCES AND NOTES

1. T. C. Orr-Weaver and J. W. Szostak, *Microbiol. Biol. Rev.* **49**, 33 (1985).
2. M. S. Meselson and C. M. Radding, *Proc. Natl. Acad. U.S.A.* **72**, 358 (1975). In both the Holliday (*3*) and Meselson-Radding models, heteroduplex DNA is formed by invasion of one parental DNA duplex by a single strand of DNA from the other parental duplex. Subsequent events lead to the formation of a cross strand structure, which can then be resolved with or without exchange of flanking markers. Gene conversion results from the repair of the heteroduplex DNA.
3. R. Holliday, *Genet. Res.* **5**, 282 (1964).
4. J. W. Szostak, T. L. Orr-Weaver, R. J. Rothstein, F. W. Stahl, *Cell* **33**, 25 (1983). In the double-stranded break model, gene conversion and recombination are initiated by a double-strand gap in one parental DNA duplex. Conversion results from the repair of this gap by copying DNA from the unbroken homolog. This structure can be resolved either with or without exchange of flanking markers.
5. J. L. Rossignol, A. Nicholas, H. Hamza, T. Langin, *Cold Spring Harbor Symp. Quant. Biol.* **49**, 13 (1984).
6. A. T. C. Carpenter, *ibid.*, p. 23.
7. D. Hurst, S. Fogel, R. K. Mortimer, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 101 (1972).
8. R. H. Borts, M. Lichten, M. Hearn, L. S. Davidow, J. E. Haber, *Cold Spring Harbor Symp. Quant. Biol.* **49**, 67 (1984).
9. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
10. M. Lichten, R. H. Borts, J. E. Haber, *Genetics* **225**, 233 (1982).
11. R. H. Borts and J. E. Haber, unpublished observations.
12. S. Fogel, R. K. Mortimer, K. Lusnak, F. Tavares, *Cold Sping Harbor Symp. Quant. Biol.* **43**, 1325 (1978).
13. P. J. Hastings, *ibid.* **49**, 49 (1984).
14. N. Rudin and J. E. Haber, in preparation.
15. R. K. Mortimer and S. Fogel, in *Mechanisms of Recombination*, R. F. Grell, Ed. (Plenum, New York, 1974), p. 263.
16. E. A. Savage and P. J. Hastings, *Current Genet.* **3**, 37 (1983).
17. L. DiCaprio and P. J. Hastings, *Genetics* **84**, 697 (1976).
18. L. S. Symington and T. D. Petes, personal communication.
19. A. L. Kolodkin, A. J. S. Klar, F. W. Stahl, *Cell* **46**, 733 (1986).
20. F. Sherman, G. R. Fink, J. B. Hicks, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
21. The Bam HI (Ba), Sal I (Sa), Sty I (St), and Nde I (Nd) sites were eliminated by filling in the 5′ overhang of restriction enzyme-digested plasmid with Klenow fragment of DNA polymerase I (*22*). The Pvu II (Pv) site was eliminated by the insertion of an Xba I linker. The Pst I (Ps) mutation is an EMS-induced point mutation (*23*). The two parents are also nonhomologous at the *URA3*-pBR322 junctions because of Xho I linkers in the *MAT*α parent and the presence of Ava I–Hind III blunt end junctions in the *MAT*α parent. *MAT-URA3*-pBR322 plasmids containing the restriction site mutations were introduced into chromosome III of Y55 by site-directed transformation (*24*). In both parents the 5′ end of the *URA3* gene is centromere proximal. All haploid strains used are either direct transformants of these two strains (or their parents) or are segregants of crosses of these strains to Y55.
22. T. F. Maniatis, E. F. Fritsh, J. Sambrook, *Molecular Cloning: A. Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
23. J. Viera and J. Messing, *Gene* **19**, 259 (1982).
24. T. L. Orr-Weaver, J. W. Szostak, R. J. Rothstein, *Methods Enzymol.* **101**, 228 (1983).

25. We thank M. Lichten and E. J. Louis for helpful discussions, A. T. C. Carpenter for critical reading of the manuscript, L. Martin-Morris for constructing some of the plasmids used, and M. Calisto for technical assistance. Supported by NIH grant GM29736, March of Dimes Foundation grant 1-968, and (to R.H.B.) a Charles H. King Trust Postdoctoral Fellowship from the Medical Foundation of Boston.

20 March 1987; accepted 20 August 1987