Different Types of Recombination Events Are Controlled by the *RAD1* and *RAD52* Genes of *Saccharomyces cerevisiae*

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ABSTRACT

Intrachromosomal recombination within heteroallelic duplications located on chromosomes *III* and *XV* of *Saccharomyces cerevisae* has been examined. Both possible orientations of alleles have been used in each duplication. Three recombinant classes, gene conversions, pop-outs and triplications, were recovered. Some of the recombinant classes were not anticipated from the particular allele orientation of the duplication. Recovery of these unexpected recombinants requires the *RAD1* gene. These studies show that *RAD1* has a role in recombination between repeated sequences, and that the recombination event is a gene conversion associated with a crossover. These events appear to involve very localized conversion of a heteroduplex region and are distinct from *RAD52* mediated gene conversion events. Evidence is also presented to suggest that most recombination events between direct repeats are intrachromatid, not between sister chromatids.

ALLELIC recombination and recombination between repeated sequences have been studied in both meiosis and mitosis in the yeast *Saccharomyces cerevisiae*. These studies have shown that recombination between repeated sequences occurs at a significant frequency (JACKSON and FINK 1981, 1985; KLEIN and PETES 1981).

The *rad52* mutation, isolated as a mutation that renders cells X-ray sensitive, profoundly suppresses meiotic recombination (GAME *et al.* 1980; PRAKASH *et al.* 1980; MALONE 1983). In mitosis there is a reduction in reciprocal exchange and gene conversion (GAME *et al.* 1980; PRAKASH *et al.* 1980; MALONE and ESPOSITO 1980). However, unequal sister chromatid exchange in the rDNA cluster and circular plasmid integration is unaffected by the mutation (PRAKASH and TAILLON-MILLER 1981; ZAMB and PETES 1981; ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). Mitotic gene conversion between delta sequences is reduced over 100-fold in *rad52-1* strains (ROTHSTEIN, HELMS and ROSENBERG 1987). Intrachromosomal gene conversion between repeated genes is reduced over 200-fold in *rad52* strains, but reciprocal exchange is unaffected (JACKSON and FINK 1981). Crossover events in an inverted repeat that originate as a gene conversion are reduced 100-fold in *rad52* strains (WILLIS and KLEIN 1987).

Examination of the types of mitotic recombination events that are sensitive to the *rad52* mutation and those few events that occur in *rad52* strains has led to the suggestion that (1) some crossovers such as sister chromatid exchange and circular plasmid integration are *RAD52* independent and (2) there is a second minor pathway for gene conversion in yeast that is *RAD52* independent (HABER and HEARN 1985; HOEKSTRA, NAUGHTON and MALONE 1986).

While these data suggest that there is more than one pathway for gene conversion in yeast, the genetic components of a second pathway have not been defined. If a second pathway promotes only a fraction of the total recombination events observed, it is difficult to observe an effect of a mutation unless one can define a class of recombination events that have a distinguishing phenotype and are primarily the result of a second pathway. The use of genetically marked duplications has permitted the recognition of such recombination events. There is a class of recombination events between direct repeated sequences similar to the events described as two recombinations (gene conversion and a subsequent reciprocal exchange) by JACKSON and FINK (1981) in *rad52-1* strains. These events are probably related to the conversion events seen in homozygous *rad52* diploids by HABER and HEARN (1985) and HOEKSTRA, NAUGHTON and MALONE (1986). In this report I show that these events are absent in *rad1* strains and suggest that there are at least two types of gene conversion events in yeast, a long patch mismatch repair mediated by *RAD52* and a short patch mismatch repair mediated by *RAD1*.

MATERIALS AND METHODS

Strains: The strains used in this study are described in Table 1. The original *rad52-7* allele was provided by D. Schild. The original *rad1*Δ::*LEU2* allele was provided by R. KEIL.

Media and growth conditions: YEPD medium, synthetic medium SD, and synthetic complete medium with bases and

TABLE 1

Strains

Strain	Genotype
JB3	*MATa his3-513::TRP1::his3-537 trp1 leu2-3,112*
312-67C	*MATα leu2-k::URA3::leu2-112 ura3-52*
329-6C	*MATα his3-537::TRP1::his3-513 leu2-3,112 trp1 ura3-52 ade6*
340-2C	*MATα leu2-112::URA3::leu2-k ura3-52 ade1-101*
350-20A	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 rad52-1*
351-5D	*MATα his3-513::TRP1::his3-537 trp1 leu2-3,112 ura3-52 rad52-1*
354-1A	*MATa leu2-112::URA3::leu2-k ura3-52 ade1-101 rad1-1*
354-9B	*MATa leu2-112::URA3::leu2-k ura3-52 ade1-101 rad1-1*
355-5B	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 leu2-3,112 ade1-101 rad1-1*
355-7B	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 ade1-101*
355-8B	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 ade1-101 rad1-1*
355-14C	*MATα his3-537::TRP1::his3-513 trp1 leu2-3,112*
356-15D	*MATα his3-513::TRP1::his3-537 trp1 ura3-52 rad1-1*
356-17B	*MATα his3-513::TRP1::his3-537 trp1 ura3-52 leu2-3,112 rad1-1*
357-28B	*MATα leu2-k::URA3::leu2-112 ura3-52 rad1-1*
359-26D	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 can1-100 rad1::LEU2 leu2-3,112*
360-2D	*MATa his3-513::TRP1::his3-537 trp1 ade1-101 ura3-52 rad1::LEU2 leu2-3,112*
363-9A	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 rad52-1*
363-11B	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 rad52-1*
363-11C	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 rad1-1*
363-13A	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 rad1-1*
363-15D	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 rad1-1 rad52-1*
363-16A	*MATα his3-537::TRP1::his3-513 trp1 ura3-52*
363-16B	*MATα his3-537::TRP1::his3-513 trp1 ura3-52 rad1-1 rad52-1*
363-24C	*MATa his3-537::TRP1::his3-513 trp1 ura3-52*
365-4A	*MATa his3-537::TRP1::his3-513 trp1 ura3-52 ade2 ade6 rad52-7*

amino acids omitted as specified were prepared as described (SHERMAN, FINK and HICKS 1986). L-Canavanine sulfate was added to synthetic medium at a concentration of 60 μg/ml and 5-fluoro-orotic acid (FOA) was added to synthetic medium at a concentration of 750 μg/ml.

Analysis of recombination rates: Recombination rates were calculated using the median method of LEA and COULSON (1948). Standard deviations of the rates were calculated according to LEA and COULSON (1948) as used by JINKS-ROBERTSON and PETES (1986). To determine each rate nine single colonies from a YEPD plate were suspended in water and appropriate dilutions were plated on YEPD, and synthetic complete minus histidine or synthetic complete minus leucine and 5-FOA plates. Independent recombinants for subsequent DNA analysis were selected by streaking colonies from YEPD plates with a toothpick onto selective medium. From these plates one colony from each streak was chosen randomly for further analysis.

DNA manipulation: Plasmid DNA was prepared as described by CLEWELL and HELINSKI (1970). Yeast genomic DNA was prepared from 5 ml YEPD cultures as described by SHERMAN, FINK and HICKS (1986). Chromosomal DNA was digested with the specified restriction enzymes as recommended by the manufacturer. DNA fragments were fractionated by agarose gel electrophoresis and transferred to nitrocellulose according to SOUTHERN (1975). ^{32}P-labeled DNA was prepared as described by FEINBERG and VOGELSTEIN (1984). Hybridization was performed in 6 × SSC, 1 × Denhardt's solution, 0.25 M NaH_2PO_4 (pH 6), 30 mM tetrasodium pyrophosphate at 65° for 18 hr. X-OMAT Kodak film was used for autoradiography.

RESULTS

Duplications used to monitor recombination: Two different duplications, each in two orientations, were constructed (Figure 1). All duplications are direct repeats. The first duplication, a 2.16-kb repeat, is at the *LEU2* locus. Strains with duplicated copies of the *LEU2* gene, each carrying a different mutation were constructed. Plasmid pBR322 sequences and the yeast *URA3* gene are located between the duplicated *LEU2* genes. One of the *LEU2* genes carries a 7-bp deletion at the *Kpn*I site, called *leu2-k*, that results in leucine auxotrophy. The second copy carries the *leu2-112* allele, a +1-bp frameshift mutation (ERNST, HAMPSEY and SHERMAN 1985; MATHISON and CULBERTSON 1985; M. CULBERTSON, personal communication). These mutations are 400–900 bp apart; the exact position of the *leu2-112* allele is not known. This duplication has been made in two orders or orientations. The orientations are numbered according to JACKSON and FINK (1981). Similar duplications of *HIS3* gene at the *HIS3* locus were made. The *HIS3* duplication is 6.1 kb. Plasmid pBR322 sequences and the yeast *TRP1* gene are located between the duplicated *HIS3* genes. One *HIS3* gene carries the *his3-513* allele, the loss of a *Kpn*I site (T. ORR-WEAVER, personal communication). The second gene carries the *his3-537* allele, loss of the most distal *Hin*dIII site (T. ORR-WEAVER, personal communication). These mutations are 316 bp apart. As in the case of the *LEU2* duplications, both orientations of the *HIS3* duplication were made.

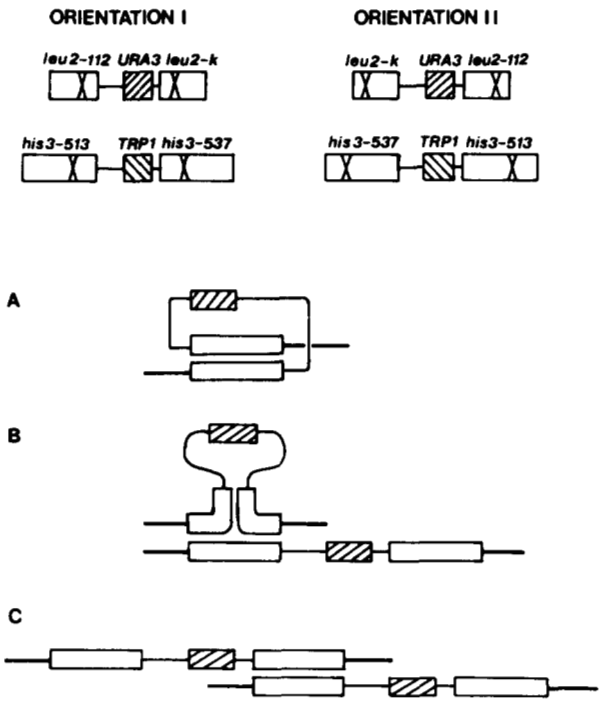

FIGURE 1.—Representation of the duplications used in this study and the types of intrachromosomal recombination events that may occur. Orientation I duplications have the alleles in the most proximal position. In orientation II the alleles are most distal. The positions of the mutations are marked by an ×. The types of recombination events that result in a change in copy number are shown below. A, Crossover (CO) between duplicated genes located on one chromatid. A chromosome with one copy of the gene and loss of the marker contained in the original duplication, a pop-out, is recovered. The event may be reciprocal, but no attempt to demonstrate this is made. The crossover may occur in association with a gene conversion, but this cannot be determined in the experiments of this report. B, Unequal gene conversion (UGC) between sister chromatids. Gene conversion can delete the loop, resulting in a single copy of the gene and loss of the selectable marker, or insert the information from the paired genes and the loop into one gene of the duplication on the sister chromatid, resulting in a triplication. C, Crossover between sister chromatids paired unequally (USCE). This can result in a single copy gene and loss of the selectable marker of the original duplication (pop-out) or a triplication.

Orientation I is formed by the proximal linear position of the mutant sites while the mutations are most distal in orientation II (Figure 1). For gene conversion events not associated with crossovers that give prototrophic segregants, the frequency of events should not be affected by orientation. In orientation I, conversions associated with crossovers, and crossovers between the alleles either within one chromatid or between sister chromatids will give a chromosome with a single wild-type *LEU2* or *HIS3* gene (Figure 1). Unequal gene conversion between one duplication on one chromatid and one member of the duplication on the sister chromatid (also called sister chromatid conversion, SCHIESTL, IGARASHI and HASTINGS 1988) can also give a single copy wild-type gene (ROTHSTEIN, HELMS and ROSENBERG 1987). Prototrophic recombinants that contain a single copy gene and have lost the marker gene between the duplicated sequences are termed Leu^+ or His^+ pop-outs. The other products of these recombination events do not give a prototrophic cell and so are not recovered. In orientation II, unequal sister chromatid exchange with or without a gene conversion should give a triplication that contains one wild-type gene. In the triplication the wild type gene should be located in the middle. The triplication can also be formed by an unequal gene conversion between sister chromatids as described above. Simple crossovers between alleles using orientation II duplications will not result in Leu^+ or His^+ pop-outs. If gene conversion is also involved only independent conversion of both alleles or conversion of one allele with a nonadjacent crossover will give Leu^+ or His^+ pop-outs from orientation II duplications.

Distribution of recombinant classes in wild-type strains: Leucine or histidine prototrophs were selected from orientation I and orientation II containing strains. Between 36 and 60 independent recombinants were examined. After scoring for pop-out events, Leu^+Ura^- or His^+Trp^- recombinants, the recombinants were analyzed by Southern hybridization using the restriction enzymes whose sites are mutated in the original duplications. Using this analysis, the recombinants were divided into four types: conversion of the centromere proximal gene without an associated crossover, conversion of the centromere distal gene without an associated crossover, pop-outs, and triplications (Figure 2).

The distribution of recombinants obtained is shown in Table 2. Half of the recombinants from the orientation I *LEU2* duplication are gene conversions without exchange and half are pop-outs. All the recombinants recovered from orientation II are gene conversions without exchange. In a second series of duplications of *LEU2* made using the *leu2-k* allele and a mutation in the *Eco*RI site in *LEU2*, *leu2-r*, the same distribution of events was observed except that 5% of the events with an orientation II construction were triplications (data not shown). The rates of Leu^+ recombinants were unchanged (7.9×10^{-6} for orientation I and 9.3×10^{-6} for orientation II).

The distribution of recombination events generating His^+ segregants is similar to those giving Leu^+ segregants, with a few exceptions. Of the recombinants from orientation I, 6% were triplications. This percentage was unchanged in orientation II. In orientation II, 25% of the recombinants were pop-outs. The higher percentage of events that are triplications and pop-outs may be related to the size of the duplication, 6.1 kb for the *HIS3* duplication and 2.2 kb for the *LEU2* duplication. It does not seem to be specific to the alleles used at *HIS3*. Examination of duplications using *his3-513* and *his3-hs21* (insertion of a *Sac*I

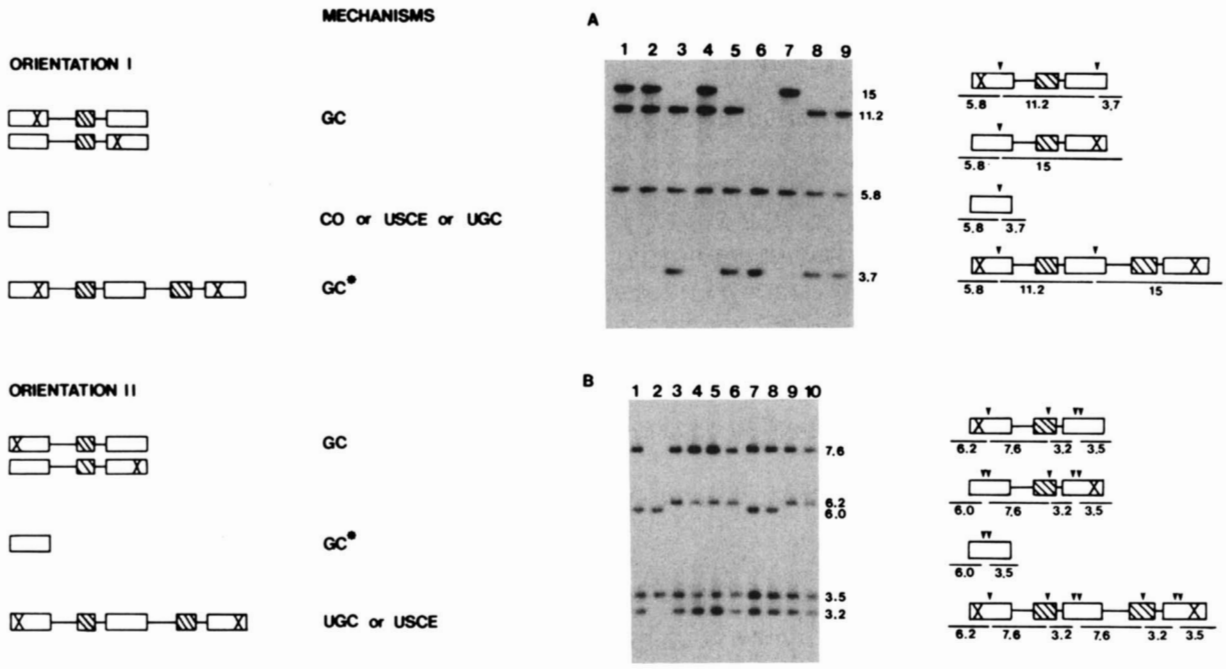

FIGURE 2.—Proposed events to generate the types of prototrophic segregants observed. Orientation I and orientation II duplications are shown. Only the simplest event that results in the diagrammed recombinant is described. Events that give a one wild-type copy and one mutant copy of the duplicated gene, with the mutant copy being in the same position in the duplication as the original duplication, and also retain the marker between the duplicated genes are called gene conversions (GC). Occasionally duplications with one wild-type gene and one mutant gene, but the mutant now being in the other copy of the gene from the original duplication are recovered. These occur at 2–4% and are interpreted as resulting from symmetric heteroduplex. In orientation I pop-outs can result from crossovers (CO) that may or may not occur in association with gene conversion, from unequal sister chromatid exchange (USCE) or from unequal gene conversion (UGC) as diagrammed in Figure 1. Triplications may result from a gene conversion that involves localized mismatch repair and is associated with a crossover (GC*). This type of gene conversion is diagrammed in Figure 3. In the case of orientation I the recombination must occur between sister chromatids to result in a triplication. Localized gene conversion associated with a crossover (GC*) occurring intrachromatid or between sister chromatids in orientation II duplications can result in a pop-out. UGC or USCE result in triplications in orientation II duplications. A and B show autoradiograms of His^+ segregants obtained with orientation II duplications. The figures to the right show the size of the bands in kilobase pairs obtained with each recombinant class from a *Kpn*I digest (upper figure) or a *Hin*dIII digest (lower figure). The diagrams are not drawn to scale. Panel A shows a *Kpn*I digest, panel B shows a *Hin*dIII digest. The 200-bp fragment from the two closely spaced *Hin*dIII sites has migrated off the gel. A, Lanes 1, 2 and 4 are triplications, lanes 3, 5, 8 and 9 are gene conversions of the *his3-513* gene, lane 6 is a pop-out and lane 7 is a gene conversion of the *his3-537* gene. B, Different samples are shown from A. Lanes 1, 7 and 8 are gene conversions of the *his3-537* gene, lane 2 is a pop-out, lanes 3, 6, 9 and 10 are gene conversions of the *his3-513* gene and lanes 4 and 5 are triplications. Plasmid YIp1 containing the *HIS3* gene was used as probe. The numbers to the right of the autoradiograms indicate the band sizes in kilobases.

linker into the *Hae*III site at position 624 of the *HIS3* coding sequence, D. LIVINGSTON, personal communication) gave similar distributions of recombinant classes to those shown in Table 2 for the *HIS3* duplications (data not shown). Triplications at *LEU2* have been observed with other allele combinations, however, they always occur at a lower frequency than at *HIS3* (see above and H. KLEIN, unpublished observations).

The unexpected observation was that, at the duplicated *HIS3* locus, a significant fraction of events were triplications from orientation I (6%) and pop-outs from orientation II (25%) (Table 2). It is difficult to explain these events by a crossover between the two alleles or by a conversion, using mismatch repair or double strand gap repair, and an adjacent crossover. However, they can be explained by the formation of heteroduplex that covers both alleles, with mismatch repair of only one heterology and an associated crossover as diagrammed in Figure 3. Mitotic segregation following DNA replication would give a prototrophic segregant. As no attempt was made to recover sectored colonies, the genotype of the other strand cannot be determined.

Sister chromatid exchange, either a simple crossover between the alleles or a conversion of one allele and an adjacent crossover, can give rise to pop-out recombinants from orientation I duplications and to triplications from orientation II duplications (Figure 2). Similar intrachromatid events can also result in pop-out recombinants with orientation I duplications. The observation that the percentage of pop-outs in orientation I (47% for *LEU2* and 44% for *HIS3*) is higher than the percentage of triplications in orientation II (0% for *LEU2* and 7% for *HIS3*) suggests that most recombinants result from intrachromatid interactions rather than sister chromatid exchanges. More complex recombination events, pop-outs in ori-

TABLE 2

Distribution of prototrophic recombinants in *RAD* strains

	Orientation I			Orientation II		
	Percent events	No. events	Rate	Percent events	No. events	Rate
LEU2 duplications						
Conversion *leu2-k*	33	12	4.6×10^{-6}	64	23	3.2×10^{-6}
Conversion *leu2-112*	20	7	2.8×10^{-6}	36	13	1.8×10^{-6}
Pop-out	47	17	2.4×10^{-6}	0		
Triplication	0			0		
Rate Leu^+			$1.4 \pm 0.3 \times 10^{-5}$[a]			$5.0 \pm 0.1 \times 10^{-6}$
HIS3 duplications						
Conversion *his3-537*	20	13	1.4×10^{-6}	28	11	1.3×10^{-6}
Conversion *his3-513*	30	20	2.2×10^{-6}	40	16	1.8×10^{-6}
Pop-out	44	29	3.2×10^{-6}	25	10	1.2×10^{-6}
Triplication	6	4	4.3×10^{-7}	7	3	3.2×10^{-7}
Rate His^+			$7.2 \pm 1.6 \times 10^{-6}$			$4.6 \pm 1.0 \times 10^{-6}$

Percent events is the percent of prototrophs examined by Southern analysis that are the result of the indicated event. The number under No. events column indicates the actual number of events in each class. The rate is derived from the rate of total Leu^+ or His^+ prototrophs multiplied by the percent events in each class. Each rate in this and all subsequent tables was calculated using nine colonies for each rate determination as described in MATERIALS AND METHODS. Strain 340-2C was used for orientation I *LEU2* duplication. Strain 312-67C was used for orientation II *LEU2* duplication. Strain JB3 was used for orientation I *HIS3* duplication. Strain 329-6C was used for orientation II *HIS3* duplication.

[a] Rates of total Leu^+ and His^+ segregants are given as the mean ± the standard deviation (JINKS-ROBERTSON and PETES 1986).

entation II and triplications in orientation I duplications, also show the same bias toward pop-outs over triplications (Table 2). This further supports the suggestion that most intrachromosomal recombination events are intrachromatid.

Distribution of recombinant classes in *rad52* strains: JACKSON and FINK (1981) have shown that intrachromosomal gene conversion events between duplicated sequences are sensitive to the *rad52-1* mutation. They also found that the frequency of triplication events was unaffected by the *rad52-1* mutation and that pop-out events showed a slight increase in *rad52-1* strains.

To examine the effect of *RAD52* on the crossover recombinant classes more closely, the *rad52-1* mutation was introduced into strains carrying the *HIS3* duplication. Both orientations of the duplication were used. To ensure that differences in His^+ rates were not the result of strain background, recombination rates were always determined for at least two spore segregants of the appropriate genotype from each cross. In addition, rates for *RAD* spores from the same cross were determined. The effect of *rad52* mutations on the pop-out and triplication recombinant classes was different from that observed by JACKSON and FINK (1981).

The distribution of recombinant classes for *HIS3* duplications in *rad52-1* strains is shown in Table 3. The rate of His^+ recombinants is reduced 10–20-fold from wild type strains (Table 2). However, conversion events not associated with exchange show a 100-fold decrease, as expected from the results of JACKSON and FINK (1981). In the orientation I containing strain, pop-out events represent a significantly larger percentage of the His^+ recombinants (86%) than in wild type strains ($P < 0.01$). However, the rate is reduced 10-fold from wild type. The percentage of triplication events is not significantly different from wild type but the rate is reduced 20-fold in the *rad52-1* strain. The orientation II containing strain shows a 100-fold reduction in gene conversion events not associated with crossovers. Pop-out events and triplications are reduced 4-fold in rate, although most of the His^+ recombinants are pop-out recombinants. Similar results were obtained using the *rad52-7* allele, a null allele of *RAD52* (see Table 3). These experiments show that specific classes of recombinants, pop-outs and triplications, are not as sensitive to the *rad52-1* mutation as the gene conversion recombinant class. More importantly, events that are dependent on crossovers were reduced, but not eliminated, in *rad52* strains, in contrast to the results of JACKSON and FINK (1981) where no effect was seen. However, some of the events that occur in the *rad52* strains cannot be explained by crossovers alone; gene conversion is required.

Distribution of recombinant classes in *rad1* strains: The *RAD1* gene of *S. cerevisiae* is involved in the excision repair process of pyrimidine dimers induced by UV irradiation (UNRAU, WHEATCROFT and COX 1971; WILCOX and PRAKASH 1981; RESNICK, BOYCE and COX 1981; REYNOLDS and FRIEDBERG 1981). *RAD1* also functions in the removal of DNA adducts that do not distort the helix (HOEKSTRA and MALONE 1986). Although no direct role in recombi-

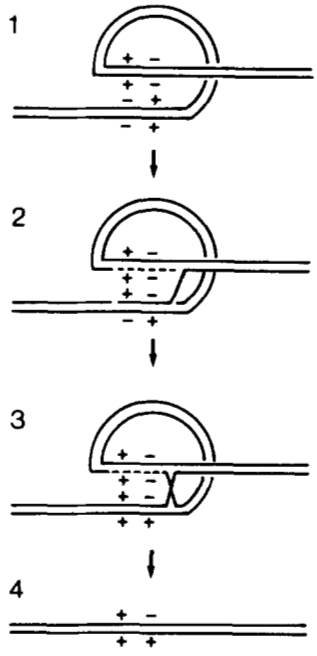

FIGURE 3.—Diagram of localized gene conversion associated with a crossover (GC*). The event depicted is shown as a mechanism to result in a prototrophic pop-out from an orientation II duplication. 1, Intrachromatid pairing of the duplicated genes. 2, Formation of heteroduplex by single strand invasion and degradation on the recipient strand and DNA synthesis on the donor strand according to MESELSON and RADDING (1975). 3, Ligation of the nicked strands, forming a Holliday junction. Heteroduplex now covers both alleles on one chromatid. 4, Mismatch repair of one of the mismatches, localized or independent mismatch repair, associated with resolution of the Holliday junction in the crossover mode, resulting in a chromosome with a single copy of the gene, loss of the marker between the duplicated genes, and heteroduplex at one of the allelic sites. DNA replication and cell division will lead to a chromosome containing only wild-type information. The scheme that is shown is the simplest mechanism requiring mismatch repair of one heterology that can lead to this intermediate. As the prototrophic segregants were selected, not screened, it was not possible to recover the sectored colonies that are predicted by this model.

nation has been found for *RAD1*, it has been possible to separate meiotic gene conversion from reciprocal exchange when meiotic cultures are irradiated at the start of meiosis (RESNICK, GAME and STASIEWICZ 1983). The triplication recombinant class in orientation I recombinants and the pop-out recombinant class in orientation II are hypothesized to result from a different type of gene conversion, one that involves localized mismatch repair. Given the reported role of *RAD1* in excision repair, a process that is thought to involve only short regions of repair synthesis (KUEMMERLE, LEY and MASKER 1981; COOPER 1982), the pattern of recombinants obtained from heteroallelic duplications was examined in *rad1-1* and *rad1*Δ::*LEU2* strains. Similar results were obtained with both alleles.

Table 4 shows the results obtained using the *LEU2* duplications. Only pop-outs were scored; the recombinants that still retained the *URA3* marker located between the duplicated genes were not characterized further. Two spore segregants that carried the *LEU2* duplication and the *rad1-1* allele were studied. In both cases, 25% of the recombinants were pop-outs, Leu^+Ura^-. Contingency chi-square tests showed that this fraction of pop-outs was significantly different from the *RAD1* fraction of 50% at $P < 0.025$ (Table 4). Using a different duplication R. KEIL, M. HOEKSTRA and B. ZEHFUS (personal communication) have also observed a reduction in recombinant pop-outs in *rad1* strains. Measurements of pop-out recombination rates assayed by Ura^- segregants recovered on 5-FOA plates showed that the *rad1-1* rates were not different from rates obtained using *RAD1* strains (*RAD1* 2.0×10^{-5}, 3.5×10^{-5}; *rad1-1* 1.6×10^{-5}, 6.9×10^{-6}), indicating that *rad1* does not affect Ura^- pop-outs.

Since the *HIS3* duplication routinely gave more triplications that the *LEU2* recombinants, His^+ recombinants were analyzed in more detail. Similar to the *LEU2* duplication, a reduction in pop-out recombinants, His^+Trp^-, in *rad1* strains compared to *RAD1* strains, was seen using orientation I duplications (Table 5). His^+Trp^+ recombinants from orientation I were not analyzed further. However, the recombinants obtained from orientation II duplications were fully analyzed. The orientation II pop-out recombinants, His^+ Trp^-, were significantly reduced in *rad1-1* strains and also in *rad1*Δ::*LEU2* strains (Table 5), to a percentage ranging from 0% to 2%. This is significantly different from that obtained in *RAD1* strains (Table 2), 25%, at $P < 0.001$. The overall rate of His^+ recombinants is not significantly different from that obtained using *RAD1* strains, but the rate of pop-out His^+ recombinants is reduced tenfold in *rad1-1* strains using orientation II duplications. This is the only recombinant class that is sensitive to *rad1* mutations. This recombinant class showed only a threefold reduction in *rad52* strains. This suggests that most of these pop-out recombinants are derived from a gene conversion (*RAD1* mediated) that is different from the *RAD52* mediated gene conversion event that accounts for approximately 90% of the overall His^+ or Leu^+ recombinants. These *RAD52* mediated recombinants are recovered in *rad1-1* strains as gene conversions without exchange and show no reduction from wild type fractions or rates (compare Tables 2 and 5). The percentage of triplications observed in *rad1-1* strains is not significantly different from that observed in *RAD1* strains ($P > 0.1$).

Distribution of recombinant classes in *rad1 rad52* strains: From the results of the studies using single mutant strains, it was anticipated that most events seen in double mutants *rad1 rad52* strains with orientation II duplications would be triplications. Since orientation II duplications of the *HIS3* gene are most informative for triplications, only this duplication was examined in the *rad1 rad52* double mutant back-

TABLE 3

Distribution of prototrophic recombinants in *rad52* strains

HIS3 duplications	Orientation I			Orientation II		
	Percent events	No. events	Rate	Percent events	No. events	Rate
Conversion *his3-537*	5	3	2.0×10^{-8}	0		
Conversion *his3-513*	4	2	1.6×10^{-8}	2	1	9.0×10^{-9}
Pop-out	86	52	3.7×10^{-7}	79	42	3.5×10^{-7}
Triplication	5	3	2.0×10^{-8}	19	10	8.4×10^{-8}
Rate His^+			$4.0 \pm 1.0 \times 10^{-7}$			$4.4 \pm 1.3 \times 10^{-7}$ [a]

The column entries are as described in Table 2. Strain 351-5D was used for the orientation I duplication and strain 350-20A was used for the orientation II duplication.

[a] The rate of His^+ obtained using strain 365-4A, which carries the *rad52-7* allele was $8.2 \pm 2.1 \times 10^{-7}$. 72% (28/39) of the prototrophs examined were pop-outs.

TABLE 4

Pop-out recombinants from *LEU2* duplications in *rad1-1* strains

LEU2 duplications	Orientation I	Orientation II
Pop-out	25% (15/59)[a]	0% (0/59)
Pop-out	25% (15/60)[a]	

Only pop-out events are listed. The number of pop-out events of the total number of prototrophs examined is listed in parentheses. Strain 354-1A was used for the top entry of the orientation I duplication and strain 354-9B was used for the second orientation I duplication. Strain 357-28B was used for the orientation II duplication.

[a] The percentage of pop-out recombinants is different from the 50% value obtained with *RAD1* strain 340-2C (Table 2) at $P < 0.025$. The remainder of the recombinants are conversion events.

ground. Two spore segregants bearing the *HIS3* duplication and both *rad* mutations were examined (Table 6). The overall His^+ rates and the rates of each class are similar. Comparison of the actual number of events using a 2 × 4 table (SNEDECOR and COCHRAN 1967) gave $P > 0.01$. This could be the result of strain background. As expected, the percentage of events that are triplications is increased and is significantly different from wild type ($P < 0.005$). Gene conversions unassociated with exchange are reduced 20–70-fold from wild type.

The effect of the double mutant background is seen more clearly when the rates of each recombinant class are examined (Table 7). In *rad52* strains the rate of triplication recombinants is independent of the *RAD1* genotype, the same reduction in rate from wild type is observed in *RAD1 rad52* and *rad1 rad52* strains.

The overall rates of His^+ recombinants from different spore segregants of one diploid are also shown in Table 6. The rates in *rad1 RAD52* strains are no different from *RAD1 RAD52* strains while the rates calculated from the *RAD1 rad52* strains are reduced tenfold. The *rad1 rad52* strains always showed a greater than tenfold reduction from wild type (Tables 6 and 7), suggesting a synergistic effect of the two mutations on the combined classes of His^+ recombinants. A synergistic effect of *rad1* and *rad52* on pop-out recombination (shown in Table 7) has also been observed by others (R. ROTHSTEIN and B. THOMAS; R. KEIL, M. HOEKSTRA and B. ZEHFUS, personal communication).

DISCUSSION

The four principal conclusions that can be drawn from these experiments are: (1) most recombination events between direct repeats are intrachromatid, not sister chromatid, (2) some sister chromatid exchanges are associated with gene conversion events, (3) *RAD1* has a role in recombination, at least in recombination between repeated sequences, that is enhanced in a *rad52* background and (4) events mediated by *RAD1* may involve localized conversion of a region of heteroduplex DNA (see model in Figure 3) and appear to be distinct from *RAD52* gene conversion events.

Examination of the prototrophic segregants recovered from duplications in both orientations in a RAD^+ background suggests that most interactions are intrachromatid, not sister chromatid. Pop-out recombinants from orientation I duplications occurred at a tenfold higher rate than triplications from orientation II duplications (Table 2). Pop-outs can result from sister chromatid or intrachromatid crossovers in orientation I duplications. Triplications must come from sister chromatid interactions (Figure 1). Unequal gene conversion between sister chromatids could generate pop-outs from orientation I duplications and triplications from orientation II duplications (ROTHSTEIN, HELMS and ROSENBERG 1987). However, if this were the major event that giving rise to the prototrophic orientation I pop-outs and orientation II triplications, then the tenfold excess of pop-outs over triplications would imply a tenfold disparity in the conversion event in favor of the deletion (pop-out) product. There are no data in yeast to support a tenfold disparity in any gene conversion event.

Most studies of sister chromatid exchange are not designed to detect gene conversion events. The experiments reported here show that some sister chro-

TABLE 5

Distribution of prototrophic recombinants in *rad1* strains

	Orientation I[a]			Orientation II[b]		
	Percent events	No. events	Rate	Percent events	No. events	Rate
HIS3 duplications						
Conversion *his3-537*		Not determined		32	19	2.5×10^{-6} [c]
Conversion *his3-513*		Not determined		47	28	3.7×10^{-6} [c]
Pop-out	20	12/60	1.4×10^{-6} [d]	2	1	1.6×10^{-7} [e]
Triplication		Not determined		20	12	1.6×10^{-6} [f]
Rate His^+			$7.0 \pm 1.2 \times 10^{-6}$			$7.9 \pm 1.5 \times 10^{-6}$
rad1Δ::LEU2 strains						
Pop-out	8	5/60		0	0/60	

The column entries are as described in Table 2. Strain 356-15D was used for the top entry of the orientation I duplication. Strain 355-8B was used for the top entry of the orientation II duplication. Strain 360-2D was used for the *rad1Δ::LEU2* orientation I duplication and strain 359-26D was used for the *rad1Δ::LEU2* orientation II duplication.

[a] A second strain, 356-17B, gave 10% pop-outs and a rate of His^+ of $6.4 \pm 1.1 \times 10^{-6}$.

[b] A second strain, 355-5B, gave 0% pop-outs and a rate of His^+ of $5.8 \pm 1.2 \times 10^{-6}$. *RAD1* spore segregants 355-7B and 355-14C gave His^+ rates of $8.5 \pm 1.5 \times 10^{-6}$ and $3.0 \pm 0.5 \times 10^{-6}$, respectively.

[c] The percentage and rate are not significantly different from those obtained with *RAD1* strains.

[d] The percentage and rate are significantly different from those obtained with *RAD1* strains at $P < 0.01$.

[e] The percentage and rate are significantly different from those obtained with *RAD1* strains at $P < 0.001$.

[f] The percentage and hence the rate are not significantly different from those obtained with *RAD1* strains.

TABLE 6

Distribution of prototrophic recombinants in *rad1 rad52* strains of orientation II duplications[a]

	Strain 363-15D			Strain 363-16B		
HIS3 duplications	Percent events	No. events	Rate	Percent events	No. events	Rate
Conversion *his3-537*	17	10	4.2×10^{-8}	10	5	1.4×10^{-8}
Conversion *his3-513*	42	25	1.0×10^{-7}	22	11	3.1×10^{-8}
Pop-out	8	5	2.0×10^{-8}	28	14	3.9×10^{-8}
Triplication	32	19	8.0×10^{-8}	40	20	5.6×10^{-8}
Rate His^+			$2.5 \pm 0.8 \times 10^{-7}$ [b]			$1.4 \pm 0.9 \times 10^{-7}$

The column entries are as described in Table 2.

[a] Experiments with orientation I *rad1 rad52* strains were not performed.

[b] *RAD1 RAD52* spore segregants 363-16A and 363-24C gave His^+ rates of $4.0 \pm 0.8 \times 10^{-6}$ and $6.6 \pm 1.2 \times 10^{-6}$, respectively. *rad1 RAD52* spore segregants 363-11C and 363-13A gave His^+ rates of $6.9 \pm 1.3 \times 10^{-6}$ and $3.9 \pm 0.8 \times 10^{-6}$, respectively. *RAD1 rad52* spore segregants 363-9A and 363-11B gave His^+ rates of $6.3 \pm 1.7 \times 10^{-7}$ and $5.8 \pm 1.5 \times 10^{-7}$, respectively.

TABLE 7

Summary of recombination rates in wild-type and *rad* strains

	RAD1 RAD52	*RAD1 rad52*	*rad1 RAD52*	*rad1 rad52*
Gene conversion	1	300×↓	1×	22–69×↓
Pop-out	1	3×↓	8×↓	30–60×↓
Triplication	1	4×↓	1×	4–6×↓
His^+	1	10×↓	1×	18–33×↓

The fold decrease from the wild-type rates is shown for orientation II *HIS3* duplications.

matid exchange events require *RAD52* function. Since mitotic gene conversion events are significantly reduced in *rad52* strains (Tables 3 and 7 and JACKSON and FINK 1981; WILLIS and KLEIN 1987; GAME *et al.* 1980; PRAKASH *et al.* 1980; MALONE and ESPOSITO 1980), a reduction in triplications from both orientation I and orientation II duplications may indicate an associated gene conversion. The sensitivity of sister chromatid exchange events to *RAD52* mutations has been interpreted as sister chromatid recombination occurring mainly through gene conversion events (FASULLO and DAVIS 1987). However, triplications always show a lower reduction in *rad52* strains than gene conversion events. This suggests that some of the triplication events may not be associated with a *RAD52* mediated gene conversion and therefore occur in *rad52* strains (Tables 3 and 6).

The triplication recombinant class from the orientation II is unaffected by the *rad1-1* mutation. This suggests that these events do not result from a *RAD1* mediated event. The remaining triplication events observed in *rad52-1* and *rad1-1 rad52-1* strains are most likely to be sister chromatid exchanges that are crossovers between the alleles without any associated gene conversion event. However, most triplication

recombinants do not result from this type of recombination since the *rad52-1* mutation results in a 4–6-fold reduction of this class (Table 7). The same rate of triplication recombinants is observed in the *RAD52* disruption strain.

Assessing the role of *RAD1* in sister chromatid exchange is difficult because the class of triplications (from orientation I) expected to show some sensitivity to a *rad1* mutation occurs at a low level (6%) even in wild type strains (orientation I, see Table 2). However, the properties of the pop-out class of recombinations in orientation II duplications suggest a contribution of *RAD1* to the sister chromatid exchanges associated with gene conversion (see Table 7 and Figure 3).

The effect of the *rad1-1* mutation on the various events studied here suggest a role for the *RAD1* product in a specific type of recombination event. Gene conversions unassociated with crossovers are not reduced in *rad1* strains, but recombination events that do involve crossovers are reduced in *rad1* strains. Pop-out recombinants that require a gene conversion event to generate a prototrophic phenotype are sensitive to *rad1* mutations. Prototrophic pop-out recombinants that may or may not be associated with a gene conversion show a twofold reduction in *rad1* strains (Table 4 and 5). These crossover products may result from intrachromatid or sister chromatid exchanges.

The effect of *rad1-1* on intrachromosomal recombination is best seen using orientation II duplications. The prototrophic pop-out class of recombinants is reduced eightfold (Table 7). No change in the frequency of gene conversion events or triplications was observed. The generation of the pop-out recombinant class from orientation II duplications is proposed to result from localized mismatch repair of a heteroduplex associated with a crossover. The sensitivity of this recombination to *rad1-1* and *rad1Δ::LEU2* is interpreted as the involvement of the *RAD1* gene product in mismatch repair events that result in gene conversion. I suggest that *RAD1* is involved only in mismatch repair of short patches, possibly in the correction of mismatches, although the heteroduplex region may be long and cover several mismatches. The gene conversion events observed by HABER and HEARN (1985) and HOEKSTRA, NAUGHTON and MALONE (1986) in *rad52-1* strains may be the result of *RAD1* mediated gene conversion events. The loss of allele specific gene conversion frequencies in *rad52-1* strains observed by HOEKSTRA, NAUGHTON and MALONE (1986) is consistent with a localized mismatch repair event, independent of sequence of the mismatch, mediated through a *RAD1* pathway. The *RAD1* mediated gene conversion events involving localized mismatch repair in a heteroduplex region may be similar to meiotic tetrads observed in *Sordaria brevicollis* (SANG and WHITEHOUSE 1983; THEIVENDIRARAJAH and WHITEHOUSE 1983) and *Sordaria fimicola* (KITANI and WHITEHOUSE 1974) where a postmeiotic segregation is separated from a crossover by a region of normal 4:4 segregation. The meiotic behavior of the duplications used in the studies reported here has not yet been examined.

The observations of the effect of *rad1* on a class of intrachromosomal recombination events has been confirmed by the analysis of *rad1 rad52* double mutants. Some recombinants may result from *RAD1* mediated or *RAD52* mediated gene conversions. The synergistic effect of *rad1* and *rad52* mutations on orientation II pop-outs in particular and on the total His^+ recombinants may be interpreted as follows. Orientation II pop-outs can result from two types of conversion events, *RAD1* or *RAD52* mediated. Some events that are mediated by *RAD1* in *RAD1 RAD52* strains may be mediated by *RAD52* in *rad1 RAD52* strains. However, in the *rad1 rad52* double mutant neither type of conversion event occurs, thus reducing the rate of pop-outs by more than the product of the reduction observed with the single mutant strains.

The initial events of recombination and the formation of heteroduplex DNA may be the same for *RAD52* and *RAD1* mediated gene conversions. I suggest that at least in the case of recombination between short direct repeats, two types of mismatch repair events can occur. The first type can result in gene conversion of a long tract to give coconversion events, is sometimes associated with reciprocal exchange, and requires the *RAD52* gene function. The second type of mismatch repair covers a short region, is preferentially associated with crossing over, and requires the *RAD1* gene product.

Are the *RAD1* mediated gene conversion events always or predominantly associated with a crossover? The only events that can be unambiguously considered *RAD1* mediated are the pop-out recombinants from orientation II. However, one can compare the rates of the gene conversion and pop-out recombinant classes from orientation II. Triplications are not considered since sister chromatid interactions appear to occur less frequently than intrachromatid events. In the *rad52-1* orientation II containing strain gene conversion events are 30-fold less frequent than pop-out events (Table 3). If both recombinants come from *RAD1* mediated conversion that is associated with crossover 50% of the time, one would expect these two classes to occur with equal frequency. The higher rate of pop-out events suggests that the *RAD1* conversions are preferentially associated with crossover. There are no data to determine whether the crossover is reciprocal in these experiments.

The experiments presented here show that *RAD1* has a substantial role in mitotic intrachromosomal recombination between repeated sequences. We have

recently found that *RAD1* is partially required for the increased crossover phenotype of a novel hyper-recombination mutation (A. AGUILERA and H. L. KLEIN, manuscript in preparation). The features of the *RAD1* mediated recombination, localized or short tract conversion and preferential association with crossovers, are consistent with the properties of recombination events observed in *rad52-1* mutants (JACKSON and FINK 1981; HABER and HEARN 1985; HOEKSTRA, NAUGHTON and MALONE 1986). Whether *RAD1* has a similar role in meiotic recombination is unknown, but the duplications described here may allow specific recombination events to be examined. Recombination data of *b2* mutants of *Ascobolus immersus* indicate that multiple gene conversion pathways operate in meiosis (HAMZA *et al.* 1986). *RAD1* mediated gene conversion may be one of these pathways in yeast.

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