Yeast Intrachromosomal Recombination: Long Gene Conversion Tracts Are Preferentially Associated With Reciprocal Exchange and Require the *RAD1* and *RAD3* Gene Products

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ABSTRACT

A yeast intrachromosomal recombination system based on an inverted repeat has been designed to examine mitotic gene conversion tract length and the association of crossing over with gene conversion as a function of the conversion tract length. Short conversion tracts are found to be preferentially noncrossover while conversion tracts longer than 1.16 kb show a 50% association with crossover. Mutation in the excision repair gene *RAD1* leads to a reduction in conversion tracts of at least 1.16 kb and a reduction in crossovers associated with conversion, regardless of the length of the conversion tract. Mutation in the excision repair gene *RAD3*, which encodes a DNA helicase, also leads to a reduction in conversion tracts of at least 1.16 kb, but has no effect on the frequency of associated crossovers. The roles of *RAD1* and *RAD3* in recombination are discussed.

GENE conversion and reciprocal exchange are intimately related in the recombination process and this close association is the cornerstone of many recombination models. Gene conversion is postulated to be one step in a series of events which yield a crossed-strand molecule. Resolution of this molecule in one mode leads to a reciprocal crossover of flanking markers while resolution in the alternative mode leads to a noncrossover chromatid that has sustained a simple gene conversion event (HOLLIDAY 1964; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Crossover and noncrossover products should thus result with equal frequency. In observations of meiotic recombinant tetrads in the fungi, values ranging from 18% to 66% of association of crossovers with gene conversion have been recorded (HURST, FOGEL and MORTIMER 1972; FOGEL, MORTIMER and LUSNAK 1981). These crossovers can account for most, if not all, meiotic exchanges (HURST, FOGEL and MORTIMER 1972). The association of reciprocal exchange with gene conversion is also seen in mitosis. Values from 10% to 55% or more have been noted (for reviews see ESPOSITO and WAGSTAFF 1981; ORR-WEAVER and SZOSTAK 1985).

It is likely that most reciprocal crossovers do not occur without the prior gene conversion step. Reciprocal exchange in mitosis and meiosis has been examined in different regions of DNA marked with heterozygous markers (BORTS and HABER 1987; WILLIS and KLEIN 1987). Over 50% of the crossovers were accompanied by a gene conversion of at least one of the heterozygous sites. Assuming a heteroduplex intermediate in the gene conversion event, this percentage association is within the predicted range if all crossovers originated from heteroduplex DNA in which mismatches could either be converted or restored.

Although these studies assume that all gene conversions are the same with respect to the potential to be resolved as crossover or noncrossover, there is evidence to suggest that shorter conversion tracts are preferentially noncrossover while longer conversion tracts show a higher association with crossovers (AHN and LIVINGSTON 1986).

The association of crossovers with gene conversion is not so strict when intrachromosomal recombination is examined between repeated sequences. Here the region of homology is short, usually on the order of 1–20 kb, and is flanked by nonhomology. Unselected meiotic events between nontandem duplications are primarily simple gene conversions that are not associated with reciprocal exchanges (KLEIN 1984; JACKSON and FINK 1985). This is thought to be the result of the limited regions of homology.

We have investigated whether all mitotic intrachromosomal gene conversion events are equal; that is, whether some types are more likely to be resolved as crossover. For these studies we have developed an intrachromosomal recombination system that is based on an inverted repeat duplication. This system permits us to make an estimate of conversion tract lengths and to determine their association with crossover. The system has been designed so that large numbers of

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events can be recovered and the nature and extent of the recombination event determined unambiguously without the necessity of performing Southern blot analysis on each recombinant. We have observed that short conversion tracts are preferentially noncrossover while conversion tracts that are at least 1.16 kb show a 50% association with crossover.

It is reasonable to expect that recombination proteins in yeast function in a multiprotein complex as suggested by ALBERTS (1984) for phage T4. As the recombination system used here allows us to distinguish simple gene conversions from conversions associated with crossovers and independently measure marker loss between direct repeats, we are able to examine the role of different genes in various aspects of intrachromosomal recombination. We have chosen genes that one might expect to affect either gene conversion tract length or resolution of the recombination intermediate into a crossover configuration.

The *RAD52* gene is required for all recombination systems studied (MALONE and ESPOSITO 1980; JACKSON and FINK 1981; WILLIS and KLEIN 1987; MALONE *et al.* 1988) with the exception of sister chromatid exchange in rDNA (PRAKASH and TAILLON-MILLER 1981; ZAMB and PETES 1981). The role of *RAD52* in the recombination system of this report is presented.

The *RAD1* and *RAD3* genes are involved in the excision of UV-induced pyrimidine dimers (for reviews see KUNZ and HAYNES 1981; FRIEDBERG 1988). *RAD1* has recently been shown to have a role in mitotic recombination (KLEIN 1988; SCHIESTL and PRAKASH 1988). The precise nature of the action of the *RAD1* gene is not clear, but has been postulated to be required for recombination events that involve conversion of heterozygous sites and resolution as crossover (KLEIN 1988). In this paper we show that *RAD1* is important for intrachromosomal crossover recombination and investigate the step at which *rad1* mutant strains are affected. The *RAD3* gene has been shown to possess ssDNA dependent ATPase (SUNG *et al.* 1987b) and DNA helicase (SUNG *et al.* 1987a) activities as predicted by the DNA sequence (REYNOLDS *et al.* 1985). Two different alleles, *rem1-1* and *rem1-2*, were isolated as hyper-rec for mitotic homolog recombination (GOLIN and ESPOSITO 1977; MONTELONE, HOEKSTRA and MALONE 1988). *In vitro* recombination studies using purified T4 proteins have shown that the *dda* DNA helicase stimulates strand exchange promoted by the uvsX protein (FORMOSA and ALBERTS 1986; KODADEK and ALBERTS 1987). If conversion tracts are a measurement of heteroduplex length, then a DNA helicase such as *RAD3* might be needed for stimulation of strand exchange. In this paper we show that the *rad3-2* allele, which has no hyper-rec phenotype, reduces the recovery of long conversion tract events.

We have also examined the role of DNA topoisomerase II, encoded by the *TOP2* gene, in intrachromosomal recombination. Type II topoisomerases unknot DNA molecules by a double-strand break (BROWN and COZZARELLI 1979; LIU, LIU and ALBERTS 1980; for a review see WANG 1985). *TOP2* mutations have little or no effect on mitotic homolog recombination (HOLM, STEARNS and BOTSTEIN 1989) or intrachromosomal pop-out recombination (CHRISTMAN, DIETRICH and FINK 1988) between direct DNA repeats but the *TOP2* gene product is required for inhibition of recombination in the rDNA tandem array (CHRISTMAN, DIETRICH and FINK 1988). Since intrachromosomal gene conversion and associated crossovers may be limited by topological constraints, we have examined whether *TOP2* is required for gene conversion and resolution of the recombination intermediate.

MATERIALS AND METHODS

Strains: Strains used are listed in Table 1. The first six strains listed were used as the original parental strains of all of the subsequent strains shown in Table 1. Strains ALD6-1 and ALD16-1 were derived from strain AHL-1D by plasmid transformation and hence are isogenic. The remaining strains of Table 1 were derived from two backcrosses of one of the first six strains of Table 1 with ALD6-1 or ALD16-1.

Media and growth conditions: Standard media were prepared as described (SHERMAN, FINK and HICKS 1986). 5-Fluoro-orotic acid (FOA) was added to synthetic complete medium (SC) at a concentration of 750 μg/ml. Strains were grown at 30°, with rotatory shaking for liquid cultures, unless otherwise indicated. Experiments with *top2-1* mutants were performed at 30° and at 32° as semipermissive temperatures for the temperature sensitive mutation *top2-1*. No differences with regard to recombination were found at the different temperatures.

Genetic analysis: Genetic analysis was performed according to published procedures (SHERMAN, FINK and HICKS 1986).

Determination of recombination rates: Experiments were performed as already described (AGUILERA and KLEIN 1988, 1989). Strains were grown on YEPD rich medium for 2–3 days. Three different strains derived from the same parental strains were analyzed for each genotype studied. For each strain, four independent colonies were analyzed for each fluctuation test. Recombination rates were calculated by the median method of LEA and COULSON (1948). The average of the three median rates is shown for each genotype in RESULTS. The standard deviation for all values was less than 30% of the average value.

His^+ recombinants were selected on complete synthetic medium lacking histidine (SC-his) and Ura^- recombinants on SC supplemented with FOA (SC+FOA). Independent His^+ recombinant colonies were isolated and scored for leucine prototrophy on SC-leu and for levels of recombination resulting in Ura^- on SC+FOA. All His^+ recombinants analyzed were scored for uracil prototrophy, to confirm that the duplication system was present. Loss of the *URA3* marker occurred in 2% of the His^+ recombinants (50/2171 His^+ recombinants). Statistical analysis (contingency chi-square) was performed both by considering the data from each strain independently and by pooling the data from all strains

TABLE 1

Strains

Name	Genotype
369-14A	*MATα trp1 his3Δ200 ura3-52*
SD117	*MATa top2-1 ade2 ura3 his3 leu2 can1 trp1*
356-12D	*MATa trp1 leu2 ura3-52 rad1-1*
373-9D	*MATa ade2-1 ura3 trp1 rad3-2*
X260-2B	*MATα ura3-52 rad52-1*
AHL1D	*MATa ade1-101 leu2Δ68 trp1 ura3-52 HIS3::leu2-r hpr1-1*
ALD6-1	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2 hpr1-1*
ALD16-1	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2 hpr1-1*
AL1644-3	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
AL644-3	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
AL644-4	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
A63-9C	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A64-6A	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A64-6B	*MATa ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A16-4A	*MATα ade1-101 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A379-5A	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A356-12B	*MATa leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
ASD17-5B	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3*
A379-5B	*MATa leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad3-2*
A379-16D	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad3-2*
A379-17C	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad3-2*
A356-5A	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad1-1*
A356-8A	*MATa leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad1-1*
A356-12A	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 rad1-1*
ASD17-1A	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 top2-1*
ASD17-11B	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 top2-1*
ASD17-19B	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 top2-1*
AL379-4C	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
AL356-2C	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
ALSD-3D	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1*
AL379-3A	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad3-2*
AL379-3B	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad3-2*
AL379-4A	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad3-2*
AL356-1A	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad1-1*
AL356-1D	*MATa leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad1-1*
AL356-11D	*MATα leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 rad1-1*
ALSD-7A	*MATα ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 top2-1*
ALSD-5B	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 top2-1*
ALSD-3B	*MATa ade2-1 leu2Δ68 trp1 ura3-52 his3-k::LEU2-leu2-r::his3Δh-URA3 hpr1-1 top2-1*

All strains listed in the table were constructed for this study with the exception of strain SD117 which was obtained from R. STERNGLANZ. The *rad1-1* and *rad3-2* alleles were obtained from strains provided by L. PRAKASH.

of the same genotype. Both analyses gave the same result and are reported below for pooled data.

DNA manipulation: Yeast genomic DNA was prepared from 5 ml YEPD cultures according to SHERMAN, FINK and HICKS (1986) and used for Southern blot analysis (SOUTHERN 1975). Plasmid DNA was isolated from *Escherichia coli* by CSCl gradient centrifugation as reported (CLEWELL and HELINSKI 1970). ^{32}P-labeled DNA plasmids were prepared according to FEINBERG and VOGELSTEIN (1984). Hybridization was performed in 6 × SSC, 1 × Denhardt's solution, 0.25 M $NaH_2P_2O_7$ at 65° for 18 hr. Linear DNA fragments were recovered directly from agarose gels and ligated using T4 DNA ligase overnight at 4° (MERTZ and DAVIS 1972). All plasmids used for subcloning steps were CsCl purified. Yeast transformation was performed according to BEGGS (1978).

Construction of strains carrying inverted and direct DNA duplications: Both an inverted and a direct DNA duplication were constructed in the 10-kb *HIS3 Eco*RI fragment of chromosome *XV* (Figure 1). This system allows the analysis of intrachromosomal recombination events that occur in an inverted repeat region of 3.0 kb. Association of crossover with a gene conversion event can be determined by the phenotype of the recombinant as explained in RESULTS. Plasmid pAA13-93 carries a 1.95-kb *Hpa*I-*Sal*I *leu2-k* fragment bearing the *leu2-k* allele which is a loss of the *Kpn*I restriction site. This fragment was inserted at the unique *Xho*I site of the 6.1-kb *Eco*RI-*Sal*I *HIS3* fragment using a *Hpa*I-*Sal*I adapter. The *Xho*I site is at the 3′ end of the *HIS3* gene outside the coding region. The plasmid copy of *HIS3* carries the *his3-k* mutation. The plasmid also carries a 1.45-kb *ARS1-TRP1 Eco*RI fragment and pBR322 sequences (AGUILERA and KLEIN 1989). Plasmid pAA13-93 was used to transform strain AHL-1D. This strain carries the *HIS3* region modified similarly to the plasmid pAA13-93 to insert a *LEU2* copy next to *HIS3*, but with a *leu2-r* allele (loss of the *Eco*RI site in *LEU2*) instead of the *leu2-k* allele, and a wild type *HIS3* gene (AGUILERA and KLEIN 1989). Leu$^+$ recombinants were isolated and studied by Southern analysis. Two transformants (ALD6-1 and

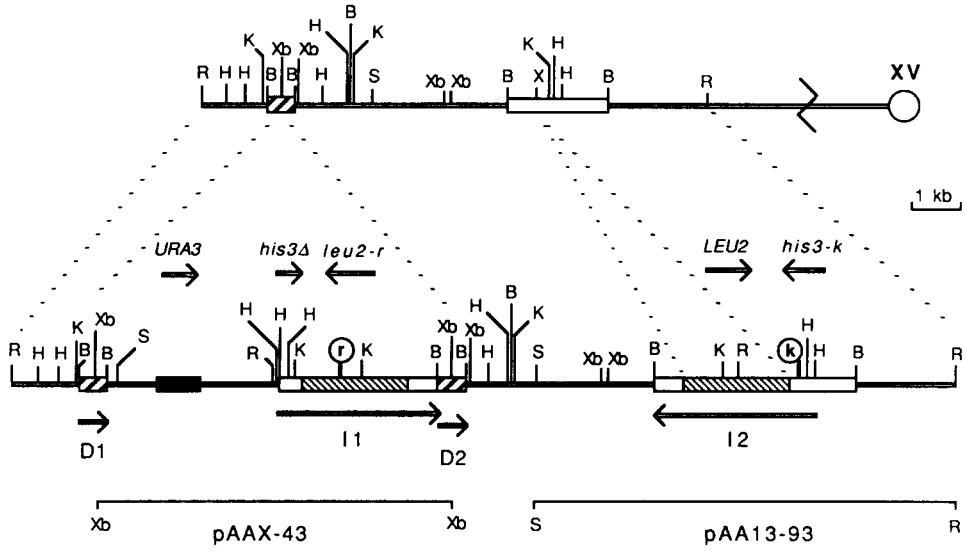

FIGURE 1.—Intrachromosomal duplication system constructed to study mitotic recombination. The wild-type 10-kb *Eco*RI *HIS3* region of chromosome *XV* is shown at the top of the figure. The large boxes indicate the two fragments of this region that were used to construct the duplication shown below. This consists of a 3.0-kb inverted duplication (repeats shown as I1 and I2) and a 0.75-kb direct duplication (repeats shown as D1 and D2). The origin of the DNA sequences is as follows: thick black line: pBR322; black box: *URA3*; box with thin diagonal lines: *LEU2*; all other fragments are from the same chromosome *XV* region as shown at the top of this figure. The mRNAs and direction of transcription of the yeast genes used to score recombination are shown. Restriction sites shown are: B: *Bam*HI; H: *Hin*dIII; K: *Kpn*I; R: *Eco*RI; S: *Sal*I; X: *Xho*I, and Xb: *Xba*I. Restriction sites removed by the Klenow reaction are shown in a circle: k: *Kpn*I, and r: *Eco*RI. The inverted repeat I1 carries a copy of *HIS3* truncated at the 5′ end and a copy of *LEU2* with the *leu2-r* allele. The inverted repeat I2 carries a wild-type *LEU2* copy and a complete copy of *HIS3* with the *his3-k* allele. The *Xho*I site lies outside of the *HIS3* coding region. At the bottom of the figure are indicated the two linearized forms of the two plasmids that were used in integrative transformation (pAAX-43) and gene replacement (pAA13-93) to generate the direct and inverted repeats. For other technical details see text.

ALD16-1) that carried a *LEU2* wild type gene and a *HIS3* gene lacking the *Kpn*I site (*his3-k*) in chromosome *XV* (Figure 1) were selected. Plasmid pAAX-43 was constructed to create both the inverted and the direct duplication shown in Figure 1. This plasmid carries a *leu2-r* gene inserted at the *Xho*I site downstream of the *HIS3* coding region. The *HIS3* gene was truncated at the *Hin*dIII site closest to the 5′ end of the gene, by partial digestion. The 3.0-kb *Bam*HI-*Hin*dIII *his3Δh::leu2-r* fragment (shown as I1 in Figure 1) was ligated to YIp5 (pBR322-*URA3*) and to a 0.75-kb *Bam*HI fragment from the downstream region of *HIS3* isolated from plasmid Sc2601 (STRUHL and DAVIS 1980). Plasmid pAAX-43 was linearized with *Xba*I, which lies in the middle of the 0.75-kb *Bam*HI fragment, and used to transform strains ALD6-1 and ALD16-1. Transformants carrying chromosome *XV* modified as shown in Figure 1 as determined by Southern blot analysis, were selected. The three strains selected (AL1644-3, AL644-3 and AL644-4) carry a 3.0 kb inverted I1-I2 repeat (*his3Δh::leu2-r–LEU2::his3-k*) and a 0.75-kb direct D1-D2 repeat which carry the *URA3* gene, pBR322 and the I1 repeat in between. They are phenotypically Ura$^+$ His$^-$ Leu$^+$. All of the strains used in the analyses reported here carried the *leu2Δ68* allele, which is a 0.6-kb deletion that covers the *Eco*RI and *Kpn*I restriction sites.

RESULTS

System used to select intrachromosomal recombination events: The construction used to select for recombination events, detailed in Figure 1, is shown with the recombination products in Figure 2. This system has been constructed so that we can determine conversion tract length and association with crossover by phenotypic scoring, thereby allowing large numbers of recombination events to be analyzed. There are two distinct repeated regions in this construct. The I regions, organized as a 3.0-kb inverted repeat, are used to study gene conversion. The 0.75-kb D regions, organized as a direct repeat, are used to determine whether a crossover has occurred between the I repeats. All the experiments described below analyze recombination events that occur in the I repeats unless otherwise indicated.

Recombinants are selected as His$^+$ prototrophs. If the conversion tract is at least 1.16 kb and is continuous, the *LEU2* allele will be converted to *leu2-r* to give a His$^+$ Leu$^-$ recombinant. If the conversion tract is short, the recombinant will have a His$^+$ Leu$^+$ phenotype. Thus the proportion of His$^+$ prototrophs that are Leu$^-$ is a measure of conversion tracts that are at least 1.16 kb (Figure 2). Given the alleles of the recombination system described here, we can only detect conversion events that are single site conversions of *his3-k* and are thus only a few base pairs or coconversion events that cover *his3-k* and the *leu2-r* alleles and are 1.16 kb. The His$^+$ Leu$^+$ recombinants measure short conversion tracts of only a few base pairs but they can extend up to 1.51 kb (1.16 + 0.35 kb). However, without additional heterozygous sites, this is not detectable. We call these short conversion

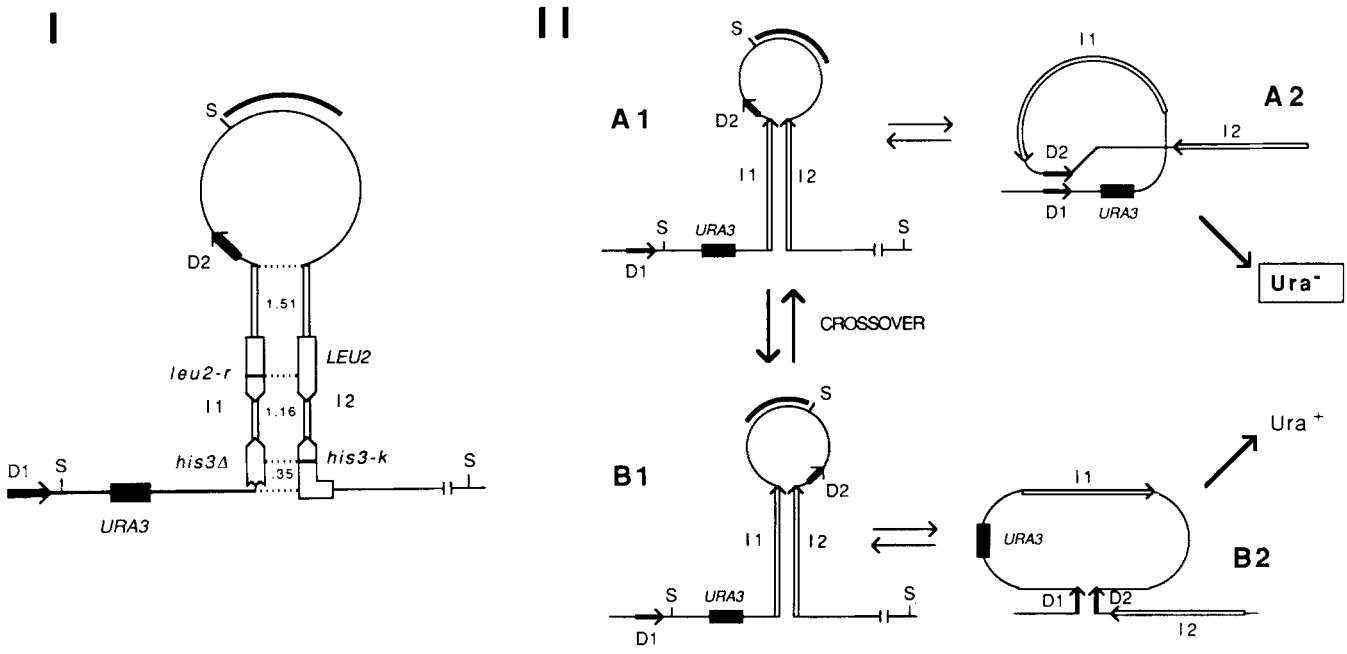

FIGURE 2.—Recombination events in the inverted repeat. I, The system shown in Figure 1 paired at the inverted repeats I1 and I2. Numbers between the paired inverted regions refer to distances in kb between the dotted lines. The phenotype of a strain harboring this duplication is His⁻ Leu⁺ Ura⁺. His⁺ recombinants are first selected. These recombinants may arise by two types of events: crossover in the lower 0.35-kb region or gene conversion covering the *his3-k* allele (whether or not associated with crossover). The His⁺ recombinants are then scored for leucine prototrophy. His⁺ Leu⁻ recombinants are events where continuous coconversion of the *his3-k* allele and *LEU2* allele has occurred (conversion tracts at least 1.16-kb and shorter than 3.0-kb). His⁺ Leu⁺ recombinants are events where no gene conversion of the *LEU2* allele has occurred (conversion tracts shorter than 1.51-kb (1.16 + 0.35-kb)). II, Different isomeric structures of this region in His⁺ recombinants not associated (A1) and associated (B1) with crossover in the inverted repeats I1 and I2. A1 and B1 show the duplication paired at the inverted repeats. These two structures are isomeric to those shown in A2 and B2, respectively, where the construct is paired at the 0.75-kb D1 and D2 direct repeats. In the original construct these repeats are in direct orientation (A2). Thus recombination events occurring in this 0.75-kb repeat will give rise to Ura⁻ pop-outs. This can easily be scored on SC + FOA plates (see Figure 3). However, when a crossover occurs between I1 and I2 to give the B1 structure, the D1 and D2 repeats that originally were in direct orientation are now in inverted orientation. The isomeric structure B2, therefore, cannot give rise to Ura⁻ events, by recombination between the D repeats. Therefore, the occurrence of a crossover between the inverted repeats I1 and I2 can be scored by Southern blot or phenotypically by determining the levels of papillation on SC + FOA (see Figure 3). S represents *Sal*I sites used for Southern analysis. The thick black line on the top of structures A1 and B1 indicates the region used as probe in Southern analysis.

events. Similarly, the coconversion events can extend from 1.16 kb to 3.0 kb, but only the 1.16-kb events are detected. We call these events conversions of at least 1.16 kb.

Gene conversion of the *his3-k* allele that is not associated with crossover will give the duplication shown in structure A1 of Figure 2. The repeated sequences D1 and D2 will remain oriented as direct repeats. These repeats have the potential to pair and generate a Ura⁻ segregant. These can be selected on 5-fluoro-orotic acid (SC+FOA) plates. Different events can lead to a deletion of an internal DNA sequence between direct repeats: intrachromatid crossover, unequal sister chromatid exchange and unequal gene conversion (ROTHSTEIN, HELM and ROSENBERG 1987; FASULLO and DAVIS 1987; MALONEY and FOGEL 1987; SCHIESTL, IGARASHI and HASTINGS 1988). In order to simplify Figure 2, only the intrachromatid crossover event is shown (structure A2), but all events listed above may contribute to Ura⁻ segregants, identified on SC+FOA plates. To avoid confusion we call Ura⁻ events pop-outs. Crossover is used only to describe reciprocal exchanges between the inverted repeats.

Crossover events that give a His⁺ segregant will generate the B1 structure of Figure 2. His⁺ Leu⁻ recombinants are the result of a conversion tract that is at least 1.16 kb. His⁺ Leu⁺ recombinants result from either: (1) conversion of the *his3-k* allele (short conversion events) or (2) crossover not associated with gene conversion in the 0.35 kb region of homology between the 5′ truncated end of the *HIS3* gene and the site of the *his3-k* allele (these crossovers may initiate anywhere along the 3.0-kb inverted repeat). When these recombinants are associated with a crossover the D repeats become oriented as inverted repeats, as shown in structure B1. Crossovers between these inverted sequences will retain the *URA3* sequence and will not grow on the selective plates containing FOA (Figure 2, B2). Ura⁻ segregants can occur from the B1 structure, but at a lower rate than from the A1 structure as two events are now required. First, a

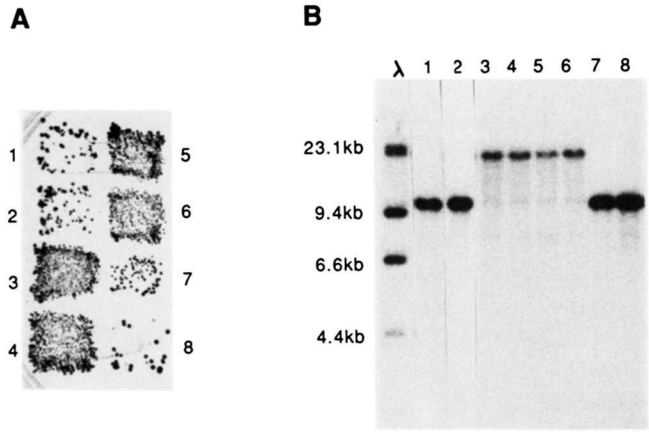

FIGURE 3.—Analysis of His$^+$ recombinants. A, Papillation on SC + FOA media observed on eight different *hpr1-1* His$^+$ recombinants. B, Southern analysis of the recombinants shown in A. A 1.4-kb *Sal*I-*Xba*I from the region in between the inverted repeats was used as probe (see Figure 2). Genomic DNA was digested with *Sal*I. When a crossover occurs between the inverted repeats, an approximately 11 kb band of hybridization is seen (recombinants 1, 2, 7 and 8). When His$^+$ recombinants occur by gene conversion not associated with crossover between the inverted repeats, a greater than 20-kb band of hybridization is seen (lanes 3, 4, 5 and 6). There is a perfect correlation between the levels of papillation on SC + FOA and the Southern analysis. In all cases high papillation on SC + FOA indicates that no crossover has occurred between the inverted repeats, and low papillation indicates the occurrence of a crossover (see Figure 2). This correlation was observed for all 149 His$^+$ recombinants analyzed by Southern blots, in both *HPR1* and *hpr1-1* strains.

crossover between the I inverted repeats will change the B1 structure into the A1 structure, placing the D repeats in direct orientation. Ura$^-$ segregants can then be generated as above.

Thus His$^+$ recombinants that are noncrossover (structure A1) should give a high frequency of papillation on SC+FOA plates. Crossover His$^+$ recombinants (structure B1) will give a low frequency of papillation on SC+FOA plates (Figure 3). The proportion of His$^+$ Leu$^-$ low papillators is therefore a measure of gene conversion tracts at least 1.16-kb that are associated with crossovers.

Gene conversion tract length and associated crossovers in wild-type and *hpr1-1* strains: Although we could distinguish high papillators from low papillators in wild-type strains, this difference is enhanced in the *hpr1-1* mutant strains. This mutation was isolated on the basis of enhanced recombination between intrachromosomal repeats and studies have shown that the intrachromatid pop-out rate is elevated 10–50 fold over wild type with no effect on gene conversion rates in intrachromosomal repeats (AGUILERA and KLEIN 1988). As shown in Figure 3, the level of papillation on SC+FOA plates is an accurate indicator that crossover between the inverted I repeats has occurred. We examined His$^+$ Leu$^+$ and His$^+$ Leu$^-$ recombinants from wild-type and *hpr1-1* strains by papillation on SC+FOA plates and by Southern analysis. We distinguished structures A1 (noncrossover) and B1 (crossover) by Southern analysis following digestion with the restriction enzyme *Sal*I which cuts once in the sequence between the I repeats and in the regions that flank the I repeats (see Figure 2). Recombinants were classified into high and low papillators (Figure 3A) and then analyzed by Southern blots (Figure 3B). Thirteen recombinants from a *hpr1-1* strain and 44 recombinants from a wild-type strain were examined. In all cases the high papillators gave the A1 structure predicted in Figure 2 while the low papillators gave the predicted B1 structure. No differences were observed between the His$^+$ Leu$^-$ and His$^+$ Leu$^+$ recombinants in terms of papillation frequency and Southern profile. Short conversion events (His$^+$ Leu$^+$) that are not crossover (high papillators) have a unique phenotype, but those short conversion tracts that are associated with crossover (His$^+$ Leu$^-$ low papillators) cannot be distinguished from crossover in the 0.35-kb region without further Southern analysis (see below).

We examined recombination in the *hpr1-1* mutant strain. We wished to determine whether the *hpr1-1* mutation affects the length distribution of gene conversions and associated crossovers in intrachromosomal recombination. No significant increase in His$^+$ recombinants was observed in the *hpr1-1* strain, but the Ura$^-$ rate was increased 16.8-fold over wild type (Table 2). The percentage of His$^+$ recombinants that are also Leu$^-$, conversions at least 1.16-kb, is unchanged (Table 2). Table 2 also shows that the percentage of gene conversions that are associated with crossover is unchanged in the *hpr1-1* strain.

The association of crossover with the length of the conversion tract was examined in wild type and *hpr1-1* strains. Table 2 shows that approximately 25% of the His$^+$ Leu$^+$ recombinants are associated with crossovers in both the wild type and *hpr1-1* strains. This class includes both short gene conversions covering the *his3-k* allele that are associated with crossover and crossovers in the 0.35-kb region. To distinguish between these two possibilities 31 independent His$^+$ Leu$^+$ crossover events (12 from *HPR1* and 19 from *hpr1-1*) were analyzed by Southern blots to determine conversion of the *his3-k* allele. DNA samples from the recombinants were digested with the *Kpn*I restriction enzyme and processed as described in MATERIALS AND METHODS. In the *HPR1* strain six cases of conversion of the *his3-k* allele and six cases of retention of the *his3-k* allele were observed. In *hpr1-1* ten cases of conversion and nine cases of no conversion were seen. Therefore in both *HPR1* and *hpr1-1* strains, the His$^+$ Leu$^+$ crossover events (low papillators) show 50% association with gene conversion. The remaining 50% are crossovers which resolve in the 0.35-kb region. These recombination events may initiate at any point

TABLE 2

Recombination rates, conversion events and crossover association in *HPR1* and *hpr1-1* strains

Genotype	Recombination rate ($\times 10^6$)		Analysis of conversion events				
			Independent His^+ events				
	His^+	Ura^-	Leu^+	Leu^-	Total	(1.16 kb GC) % Leu^-	χ^{2a}
HPR1	4.4	19.0	293	159	452	35.2	
hpr1-1	10.0	320.0	270	189	459	41.2	3.22

	Association of crossovers with gene conversion tract length						
	Leu^+ (short GC)			Leu^- (1.16 kb GC)			
	Simple GC	With CO[b]	% CO[b]	Simple GC	With CO	% CO	χ^{2c}
HPR1	177	55	23.7	82	63	43.4	15.27*
hpr1-1	144	55	27.6	70	84	54.5	25.21*

Strains used were: for *HPR1*: A63-9C, A64-6A, A64-6B and A16-4A and for *hpr1-1*: AL1644-3, AL644-3 and AL644-4. For each genotype three to six different colonies from each strain were used for a fluctuation test. A median rate was obtained for each strain. The average of the median values obtained for each genotype is shown. His^+ recombinants were selected on SC-his and Ura^- recombinants on SC + FOA. Each His^+ selected was independent and was derived from a single colony grown on YEPD and scored for prototrophy on SC-leu after His^+ selection. Loss of the *URA3* marker occurred in less than 2% of the His^+ recombinants as scored on SC-ura plates. The occurrence of a crossover in the course of the recombination event that gave rise to His^+ Leu^+ and His^+ Leu^- recombinants was determined by examining papillation on SC + FOA for each independent recombinant. The scoring of crossovers in 44 *HPR1* recombinants and 13 *hpr1-1* recombinants was confirmed by Southern analysis (see Figure 3). Statistical analysis was performed in two ways: by considering the data obtained with each strain separately and by pooling the data for each genotype. Since the number of events analyzed for each strain is similar and the results showed little variation for each strain, similar results were obtained from both methods of considering the data. The results from the pooled data for each genotype are shown in this table and in Tables 3 and 4.

[a] χ^2 value refers to the comparison of the ratio of Leu^+:Leu^- between *HPR1* and *hpr1-1* strains.

[b] These values are not corrected for the His^+ Leu^+ events that are crossovers unassociated with gene conversion of the *his3-k* allele. Approximately half of the "with CO" class are not associated with detectable conversions and would reduce the %CO in *HPR1* and *hpr1-1* to 13.7% and 16.3%, respectively.

[c] χ^2 value refers to the comparison of the ratio of simple GC events:with CO events between the Leu^+ and Leu^- classes for each genotype. When the difference between the compared rates is significant ($P < 0.05$), an asterisk is shown. When the values are compared between *HPR1* and *hpr1-1* no significant differences are seen ($\chi^2 = 0.68$ for Leu^+ events; $\chi^2 = 3.25$ for Leu^- events).

along the 3.0-kb inverted repeat. This class does not show gene conversion of the *his3-k* allele and therefore is excluded from our measurement of crossover associated with gene conversion. Thus only half of the His^+ Leu^+ crossovers (of a total of 55 each for *HPR1* and *hpr1-1* strains in Table 2) are from gene conversions of the *his3-k* allele, giving a 13.7% association of short gene conversion tracts with crossovers in *HPR1* and a value of 16.3% in *hpr1-1*.

His^+ Leu^- recombinants showed an approximately 50% association with crossover. Because the *HIS3* and *LEU2* alleles are 1.16-kb apart, these conversion events include at least 1.16-kb, assuming a single continuous event. Studies examining gene conversion lengths through the use of multiple heterozygous markers and Southern analysis have concluded that most gene conversion events are continuous (BORTS and HABER 1987; JUDD and PETES 1988; SYMINGTON and PETES 1988). Indeed, using the *HIS3::LEU2* region in a plasmid-chromosome recombination system, we have found that in 98 gene conversion events spanning more than 1.2-kb, all events in both *HPR1* and *hpr1-1* strains are continuous (AGUILERA and KLEIN 1989). There is no difference between the wild type and *hpr1-1* strain; however, the percentage association with crossover is significantly different than the values observed with the shorter conversion tract events. Preferential association of long conversion tracts with crossovers has been observed with repeats located extrachromosomally on a plasmid (AHN and LIVINGSTON 1986).

These results confirm previous observations of the *hpr1-1* mutation on pop-out recombination and gene conversion (AGUILERA and KLEIN 1988). The data on conversion tract length and association with crossovers show that the *hpr1-1* mutation does not affect either the length of the conversion tract or the association with crossover. The *hpr1-1* allele leads to an increase only in crossovers, not in gene conversions associated with crossovers.

In *HPR1* strains the *rad52-1* mutation reduces the frequency of His^+ 50-fold (rate 8.7×10^{-8}) and the frequency of Ura^- tenfold (rate 1.7×10^{-6}) (data not shown). These values are similar to those reported for direct repeat mitotic recombination (KLEIN 1988). Similar results were obtained with *hpr1-1 rad52-1* strains.

Gene conversion tract length and associated crossovers in *rad1*, *rad3* and *top2* strains: The longer conversion tracts show a higher association with crossovers (Table 2). It is possible that the shorter conversion tracts are topologically constrained from being resolved as crossover. Therefore we examined strains defective in intrachromosomal crossover recombination or DNA enzymes potentially involved in opening the DNA helix for the distribution of gene conversion events and the association of these events with crossovers. No hyperrecombination phenotype has been associated with the *rad3-2* allele used in these studies (MONTELONE, HOEKSTRA and MALONE 1988). We also examined double mutants with *hpr1-1* and either *rad1-1*, *rad3-2* or *top2-1* to look for possible interactions between these gene products.

The data of Table 3 show that none of the mutations studied, either *rad1-1*, *rad3-2* or *top2-1* affects the rate of His^+ recombinants. However, the rate of

TABLE 3

Recombination rates and conversion events in *HPR1*, *hpr1-1*, *rad1-1*, *rad3-2* and *top2-1* strains

Genotype	Recombination rate (×10⁶) His⁺	Ura⁻	Independent His⁺ events Leu⁺	Leu⁻	Total	(1.16 kb GC) % Leu⁻	χ^{2a}
HPR1	9.4	22.0	67	58	125	46.4	
HPR1 rad1-1	6.8	3.8[b]	100	21	121	17.4	22.48*
HPR1 rad3-2	14.0	46.0	87	37	124	29.8	6.55*
HPR1 top2-1	4.6	17.4	60	46	106	43.4	0.11
hpr1-1	20.0	183.0	73	55	128	43.0	
hpr1-1 rad1-1	8.7	33.3[b]	354	112	466	24.0	16.89*
hpr1-1 rad3-2	17.5	276.3	110	36	146	24.7	9.50*
hpr1-1 top2-1	6.2	214.7	103	60	163	36.8	0.89

Strains used were: for *HPR1*: A379-5A, A356-12B and ASD17-5B; for *HPR1 rad1-1*: A356-5A, A356-8A and A356-12A; for *HPR1 rad3-2*: A379-5B, A379-16D and A379-17C; for *HPR1 top2-1*: ASD17-1A, ASD17-11B and ASD17-19B; for *hpr1-1*: AL379-4C, AL356-2C and ALSD-3D; for *hpr1-1 rad1-1*: AL356-1A, AL356-1D and AL356-11D; for *hpr1-1 rad3-2*: AL379-3A, AL379-3B and AL379-4A; for *hpr1-1 top2-1*: ALSD-3B, ALSD-5B and ALSD-7A. For each genotype 3 different colonies from each strain were used for a fluctuation test with each strain. A median rate value was obtained for each strain. The average of the median values obtained for each genotype is shown. His⁺ recombinants were selected on SC-his and Ura⁻ recombinants on SC + FOA. Each His⁺ recombinant that was selected was independent and was derived from a single colony grown on YEPD and scored for leucine prototrophy on SC-leu after His⁺ selection. Statistical analysis was performed as described in Table 2.

[a] χ^2 value refers to the comparison of the ratio of Leu⁺:Leu⁻ between each mutant and the related wild-type parent, either *HPR1* or *hpr1-1*. When the difference between the compared ratios is significant ($P < 0.05$), an asterisk is shown. When *HPR1* and *hpr1-1* strains are compared no significant differences are obtained: *RAD1 RAD3 TOP2* $\chi^2 = 0.18$, *rad1-1* $\chi^2 = 2.08$, *rad3-2* $\chi^2 = 0.67$, *top2-1* $\chi^2 = 0.91$.

[b] A significant reduction in the rate of Ura⁻ pop-out events is seen only in *rad1-1* strains.

Ura⁻ pop-outs is reduced by the *rad1-1* mutation in *HPR1* and *hpr1-1* strains.

Although the rate of His⁺ recombinants is unchanged in *rad1-1* and *rad3-2* mutants, the percentage of His⁺ events that are Leu⁻, that is, those that arise from long conversion tracts, is significantly reduced, from 43–46% in *RAD* backgrounds to 17–29% in the *rad* mutant backgrounds (Table 3). This is seen in both wild type and *hpr1-1* backgrounds. The *top2-1* mutation has no detectable effect on conversion tract length. These results suggest that *RAD1* and *RAD3* are required for complete extension of the conversion tract.

To determine whether the percentage crossover associated with gene conversion was altered by *rad* and *top* mutations, papillation on SC+FOA plates was examined. Neither *rad3-2* nor *top2-1* affected the percentage crossover associated with gene conversion (Table 4). However, the percentage crossover was significantly reduced by two- to fivefold in *rad1-1* mutants (Table 4). This effect is seen in *hpr1-1* and *HPR1* strains. Since *rad1-1* reduces crossovers we were concerned that the papillation test was not an accurate assay for crossover. Therefore we examined all of the *HPR1 rad1-1* His⁺ recombinants and 40 *hpr1-1 rad1-1* His⁺ recombinants by Southern blots to verify the classification into crossover or non-crossover. Southern blotting and papillation assays gave concordant results in all cases. The reduction in associated crossovers in *rad1-1* strains suggests that *RAD1* is required at some step of crossover resolution.

DISCUSSION

In previous studies of mitotic intrachromosomal recombination the use of heteroallelic duplications to select prototrophic recombinants did not allow a distinction to be made between events that were gene conversions associated with crossovers and simple crossovers (JACKSON and FINK 1981; FASULLO and DAVIS 1987; LICHTEN, BORTS and HABER 1987; KLEIN 1988). In this report we are examining true association of crossover with gene conversion since the initial event selected must be a gene conversion with the exception of crossovers in the 0.35-kb region of the duplication. These crossovers can be excluded from determinations of conversion associated crossovers because no conversion of the *his3-k* allele has occurred. When the data for wild type gene conversions are pooled (from Table 2) we find for a gene conversion event in a repeat of 3.0-kb a maximum value of 26% associated crossovers. We have noted that longer gene conversion tracts show a higher associated crossover value of 43.4–54.5% while shorter tracts show 13.7–16.3% associated crossovers (Table 2). AHN and LIVINGSTON (1986) obtained similar results examining recombination in an extrachromosomal plasmid. As suggested by the data of AHN and LIVINGSTON (1986) the fraction of conversions that are also crossovers increases linearly with conversion tract length to reach 50% maximum for tracts of 1.5-kb to 2.0-kb. This is consistent with the observations reported for a plasmid chromosome recombination system where conversion tracts longer than 1.16-kb show a plateau level of association with crossing over (AGUILERA and KLEIN 1989). The reduced level of association (23%) in those experiments could be explained by a selective disadvantage of the *TRP1* marker in single copy when inserted into the chromosome at *HIS3*.

It is unlikely that the conversion events of this study that are at least 1.16-kb are discontinuous since physical determinations of gene conversion length by Southern analysis of multiple heterologies have shown that most events in meiosis (BORTS and HABER 1987; SYMINGTON and PETES 1988) and mitosis (AHN and LIVINGSTON 1986; JUDD and PETES 1988; AGUILERA and KLEIN 1989) are continuous.

TABLE 4

Association between gene conversion tracts and crossovers in *HPR1*, *hpr1-1*, *rad1-1*, *rad3-2*, and *top2-1* strains

Genotype	Leu$^+$ (short GC)				Leu$^-$ (1.16 kb GC)			
	Simple GC	With CO[a]	% CO[a]	χ^{2b}	Simple GC	With CO	% CO	χ^{2b}
HPR1	54	15	21.7		22	29	56.9	
HPR1 rad1-1	32	0	0	6.53*	18	2	10.0	10.97*
HPR1 rad3-2	70	17	19.5	0.02	16	13	44.9	0.65
HPR1 top2-1	44	12	21.4	<0.01	20	26	56.5	<0.01
hpr1-1	47	18	27.7		30	25	45.5	
hpr1-1 rad1-1	218	12	5.2	25.61*	45	14	23.7	5.04*
hpr1-1 rad3-2	82	26	24.1	0.12	21	15	41.7	0.02
hpr1-1 top2-1	72	30	29.4	<0.01	24	34	58.6	1.47

Strains used are those from Table 3. The events analyzed are the His$^+$ events presented in Table 3. The occurrence of a crossover associated with the His$^+$ Leu$^+$ and His$^+$ Leu$^-$ recombinants was determined by scoring papillation on SC + FOA plates for each recombinant. The crossover classification of 52 recombinants of the *HPR1 rad1-1* genotype and 40 recombinants of the *hpr1-1 rad1-1* genotype were confirmed by Southern analysis (see Figure 3). Statistical analysis was performed as described in Table 2.

[a] These values are not corrected for the His$^+$ Leu$^+$ crossover recombinants that are not associated with gene conversion. The values thus are maximum numbers.

[b] χ^2 value refers to the comparison of the ratio of simple GC events:with CO events between each mutant and the related wild-type parent, either *HPR1* or *hpr1-1*. When the difference between the compared ratios is significant ($P < 0.05$), an asterisk is shown. No significant differences were found between *HPR1* and *hpr1-1* strains: *RAD1 RAD3 TOP2*$\chi^2 = 0.36$ for Leu$^+$ recombinants and 0.96 for Leu$^-$ recombinants; *rad1-1*$\chi^2 = 0.76$ for Leu$^+$ and 0.79 for Leu$^-$: *rad3-2*$\chi^2 = 0.34$ for Leu$^+$ and <0.01 for Leu$^-$; *top2-1*χ^2 <0.01 for both Leu$^+$ and Leu$^-$.

The system used in the studies reported here can only distinguish between conversion tracts that are short, or longer than or equal to 1.16-kb. However this is an arbitrary distinction and is not used to imply a different mechanism or event. Although technically the His$^+$ Leu$^+$ events are the result of a conversion of a few base pairs, the conversion tract can range from a few base pairs to 1.51-kb (1.16-kb + 0.35-kb). The cases where the tract is longer than 1.51-kb are not considered since this would imply discontinuous events with restoration of the wild-type sequence at the *leu2-k* site. This type of event has been reported to be very rare in yeast (AHN and LIVINGSTON 1986; BORTS and HABER 1987; WILLIS and KLEIN 1987; SYMINGTON and PETES 1988; JUDD and PETES 1988). We believe that single site conversions and coconversions come from a single recombination mechanism that gives a range of conversion tract lengths, as has been clearly shown by several groups (AHN and LIVINGSTON 1986; BORTS and HABER 1987; SYMINGTON and PETES 1988).

If there is a linear relationship between conversion tract length and the percentage associated crossovers, then conversion tracts that are only slightly shorter than our cutoff point of 1.51-kb (1.16 + 0.35-kb) would show a 50% association with crossover as predicted from the isomerization and resolution of a crossed-strand structure in models of recombination (SIGAL and ALBERTS 1972; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983) while those substantially shorter than 1.51-kb (1.16 + 0.35-kb) show a lower association with crossovers, leading to an overall association of 13.7–16.3% for short conversions. Thus short conversion tracts are preferentially noncrossover. These conversions would either come from a second conversion pathway that is not mechanistically linked to crossovers or from the major crossover associated conversion pathway, but are prohibited from resolution as crossover. If intrachromosomal gene conversion events are mainly short conversion tracts, then most will be noncrossover as has been observed in meiosis (KLEIN 1984).

The suggestion of CARPENTER (1984) that some conversion events are repair events that are not processed such that they have the potential to extend heteroduplex DNA and be resolved as crossovers, while other conversion events are further processed to eventually become conversion events that show 50% association with crossing over, may explain our observations. According to this hypothesis most intrachromosomal gene conversion events would be short tract repair events that never have the potential to be resolved as crossover.

The *hpr1-1* mutation leads to an increase in intrachromosomal pop-outs, seen as Ura$^-$ recombinants, as previously reported (AGUILERA and KLEIN 1988). *hpr1-1* has also been shown to enhance crossover in a 360-bp inverted repeat 13-fold (AGUILERA and KLEIN 1989). This effect is specific to crossing over. Using the duplication system of this paper where a gene conversion (His$^+$) event is selected first, there is no difference in either the distribution of conversion events or associated crossovers between *HPR1* and *hpr1-1* strains. This suggests that the *hpr1-1* mutation does not affect events that successfully complete a gene conversion. Since *hpr1-1* does not affect crossing over associated with conversion of *his3-k*, we do not expect it to affect crossovers that are associated with

a restoration event at the *his3-k* allele. There may be a second pathway of recombination that is manifest as intrachromosomal crossovers and that this pathway is specifically affected by the *hpr1-1* mutation.

As previously reported (JACKSON and FINK 1981; KLEIN 1988) we find that the *rad52-1* mutation, while affecting both gene conversion and crossover events, leads to a greater decrease in gene conversion events (His^+) than pop-outs (Ura^-). For this reason, we could not use the papillation assay to examine associated crossovers in the few gene conversion events that were seen. Since so few gene conversions occur, the data to distinguish between short and long conversion tracts were not significant.

Three effects of the *rad1-1* mutation on recombination can be seen using the duplication system of this report. First, if only pop-out recombinants (Ura^-) are considered, *rad1-1* leads to reduction in the rate in both wild-type and *hpr1* strains. Second, *rad1* mutation leads to a significant reduction in long conversion tract events as seen by a reduction in the percentage of Leu^- segregants among the His^+ recombinants. Third, *rad1* mutation leads to a significant decrease in crossovers associated with both short tract and long tract conversion events. The latter two effects account for the reduction in Ura^- recombinants. The requirement of *RAD1* for the wild type level of Ura^- pop-outs suggests that some of these events are generated by intrachromatid crossover.

This could be explained by a role of *RAD1* in extension of the conversion intermediate, possibly heteroduplex DNA. If there is a minimum length of conversion tract so that it can be resolved as crossover and *RAD1* is required for extension of a heteroduplex to achieve this length, this would explain the observed effects of the *rad1-1* allele reported here. Failure to extend the heteroduplex or conversion intermediate will result in shorter conversion tracts. These are preferentially noncrossover, hence a reduction in Ura^- crossover recombinants will be seen. However this does not explain the reduction in associated crossovers in the long conversion tracts that do occur in the *rad1-1* mutant strains. This suggests a second role for the RAD1 protein in the isomerization or resolution step.

SCHIESTL and PRAKASH (1988) have reported that *rad1* mutations lead to a reduction in intrachromosomal recombination between direct repeats. *rad1* mutation has also been shown to reduce the rate of recovery of a class of intrachromosomal recombination events that require short localized conversion in heteroduplex longer than 0.35 kb that are associated with crossover (KLEIN 1988). In these experiments the recombination events that were reduced by *rad1* mutation had to result from a short conversion tract. Long conversion tract events could not be seen using the heteroallelic duplications. The bias in selecting recombinants is not present in the experiments reported here. In this system there is an extended basis for a conversion intermediate, either heteroduplex formation or a double stranded gap and a different topology is present as well. The data presented in Tables 3 and 4 show that *RAD1* is required for events arising from extended heteroduplex or conversion intermediate and isomerization for resolution as crossover. A similar role for *RAD1* could explain previous results (KLEIN 1988; SCHIESTL and PRAKASH 1988).

We have found that the *RAD3* is also involved in mitotic recombination. Mutation in this gene leads to a reduction in long conversion tract events with no effect on crossovers. The *RAD3* gene has been shown to have DNA helicase and ATPase activities (SUNG *et al.* 1987a, b). The T4 *dda* helicase is not required for an *in vitro* strand exchange reaction but stimulates strand exchange promoted by the uvsX protein (KODADEK and ALBERTS 1987). The RAD3 protein has been suggested to unwind the DNA duplex during DNA replication and repair (SUNG *et al.* 1987a). Such a function would explain why a null allele of *RAD3* is lethal (NAUMOVSKI and FRIEDBERG 1983). In this report we suggest that *RAD3* may have a similar role in recombination, either in unwinding the DNA duplex to allow extension of heteroduplex DNA or conversion intermediate or in unwinding the DNA heteroduplex to allow DNA repair. Possible roles for *RAD1* and *RAD3* in mitotic recombination are as accessory factors required for extension of a recombination initiation complex to long conversion tracts or long heteroduplex.

Mutation in *TOP2*, in addition to mutation in *TOP1*, enhance recombination with the rDNA cluster of yeast (CHRISTMAN, DIETRICH and FINK 1988). We found no effect on *top2* mutation on intrachromosomal recombination in any of the parameters that we have been able to assay.

We have presented an *in vivo* intrachromosomal recombination system that has allowed us to study the effects of known mutations on conversion tract length and association of crossovers with gene conversion through the analysis of a large number of independent recombination events. We have been able to demonstrate novel roles for the *RAD1* and *RAD3* gene in mitotic recombination. *RAD1* and *RAD3* may have a role in all types of mitotic recombination. However, the effect of mutations in these genes may not be great enough to be detected when recombination between homologous chromosomes is studied. Other factors such as having large regions of homology present may mask the effects of these *rad* mutations. Only when recombination between regions of limited homology such as intrachromosomal recombination is studied is the role of these genes evident.

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