Genetic Control of RNA Polymerase I-Stimulated Recombination in Yeast

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ABSTRACT

We examined the genetic control of the activity of *HOT1*, a *cis*-acting recombination-stimulatory sequence of *Saccharomyces cerevisiae*. Mutations in *RAD1* and *RAD52* decrease the ability of *HOT1* to stimulate intrachromosomal recombination while mutations in *RAD4* and *RAD50* do not affect *HOT1* activity. In *rad1Δ* strains, the stimulation of excisive recombination by *HOT1* is decreased while the rate of gene replacement is not affected. In *rad52-8* strains the ability of *HOT1* to stimulate both excisive recombination and gene replacement is decreased. All of the recombinants in the *rad52-8* strains that would be categorized as gene replacements based on their phenotype are diploids apparently derived by endomitosis and excisive recombination. Studies on *rad1Δ rad52-8* strains show that these mutations interact synergistically in the presence or absence of *HOT1*, resulting in low levels of recombination. The rate of gene replacement but not excisive recombination is stimulated by *HOT1* in *rad1Δ rad52-8* strains. Taken together, the results show that *HOT1* stimulates exchange using multiple recombination pathways. Some of the activity of *HOT1* is *RAD1*-dependent, some is *RAD52*-dependent, and some requires either *RAD1* or *RAD52* as suggested by the synergistic interaction found in double mutant strains. There is also a component of *HOT1* activity that is independent of both *RAD1* and *RAD52*.

THE rate of homologous recombination is not uniform throughout the genome of an organism. One factor that affects the rate of mitotic exchange is transcription. KEIL and ROEDER (1984) identified a *cis*-acting recombination hotspot, *HOT1*, that stimulates mitotic recombination when inserted at novel locations in the yeast genome. This hotspot is part of the ribosomal DNA (rDNA) repeat unit of yeast. The fragment that stimulates recombination contains the enhancer (ELION and WARNER 1984, 1986) and transcription initiation site (KLEMENZ and GEIDUSCHEK 1980; BAYEV *et al.* 1980) for the 35S rRNA. Recombination is stimulated only when the fragment is oriented such that RNA polymerase I transcription initiated in the fragment could proceed across the recombining sequences. Subcloning (VOELKEL-MEIMAN, KEIL and ROEDER 1987) and linker insertion (STEWART and ROEDER 1989) studies showed that approximately the same regions are required both for highly efficient, properly initiated transcription and for recombination hotspot activity. In addition, insertion of the transcription termination site (KEMPERS-VEENSTRA *et al.* 1986) between *HOT1* and the recombining sequences abolishes the recombination-stimulatory activity of *HOT1* (VOELKEL-MEIMAN, KEIL and ROEDER 1987). THOMAS and ROTHSTEIN (1989a) found that RNA polymerase II transcription also stimulates mitotic recombination between directly repeated sequences.

Transcription and recombination are also thought to be associated for some site-specific and meiotic recombination events. In yeast, only transcriptionally active copies of the three mating-type loci are cleaved by the *HO* endonuclease (KLAR, STRATHERN and ABRAHAM 1984), permitting them to function as recipients during the switching event. In mammalian cells rearrangement of immunoglobulin and T cell receptor genes is enhanced by transcription (BLACKWELL *et al.* 1986; YANCOPOULOS *et al.* 1986; SCHLISSEL and BALTIMORE 1989). BLACKWELL *et al.* (1986) postulated that transcription may alter the accessibility of these genes for rearrangement. NICOLAS *et al.* (1989) showed that the promoter region of the yeast ARG4 gene contains an initiation site for meiotic gene conversion. Double-strand breaks occur in this region at the time of recombination as well as near two other promoters that were studied (SUN *et al.* 1989).

Numerous mutations in yeast affect DNA recombination and repair. Analysis of strains containing two or more of these mutations defines three epistasis groups based on sensitivity to ultraviolet (UV) or ionizing radiation or to mutagenic chemicals (reviewed by HAYNES and KUNZ 1981). The epistasis groups have been termed (1) error-free excision repair of pyrimidine dimers or the *RAD3* group, (2) double-strand-break repair or the *RAD52* group, and (3) error-prone repair or the *RAD6* group. We studied

the effects of mutations in genes from the *RAD3* and *RAD52* groups on *HOT1* activity.

MONTELONE, HOEKSTRA and MALONE (1988) found that mutations in either *RAD1* or *RAD4*, members of the *RAD3* group, increase the frequency of interchromosomal crossing over but not gene conversion. They also found that a mutation in *RAD1* or *RAD4* abolishes the enhanced mitotic gene conversion phenotype of *rem1*, an allele of *RAD3*, but not the increased level of crossing over in *rem1* strains. Mutations in *RAD1* reduce the rate of certain intrachromosomal recombination events during mitosis (SCHIESTL and PRAKASH 1988; KLEIN 1988; THOMAS and ROTHSTEIN 1989b). The precise roles of the *RAD1* and *RAD4* products in recombination is unclear but it has been suggested that they are involved in forming or processing certain intermediates for recombination-repair (MONTELONE, HOEKSTRA and MALONE 1988; SCHIESTL and PRAKASH 1988; KLEIN 1988).

Genes of the double-strand-break repair group are also involved in mitotic recombination. Numerous reports document that *rad52* mutations reduce the rate of several forms of mitotic recombination. These include interchromosomal (PRAKASH *et al.* 1980; GAME *et al.* 1980; MALONE and ESPOSITO 1980) and intrachromosomal (JACKSON and FINK 1981) mitotic recombination. In addition, *RAD52* is required for mating-type switching (MALONE and ESPOSITO 1980), and repair of double-strand breaks (RESNICK and MARTIN 1976). MALONE and ESPOSITO (1981) and MALONE (1983) found that mutations in the *RAD50* gene, another member of the *RAD52* group, increase the frequency of mitotic interchromosomal recombination.The mechanism by which RNA polymerase I transcription stimulates recombination is not known. To determine whether *HOT1*-stimulated mitotic recombination requires at least some of the same gene products as other mitotic exchange, we have studied the effects on *HOT1* activity of several *rad* mutations. The results indicate that a number of exchange pathways are involved in producing the recombination-stimulatory activity of *HOT1* and that at least some of these pathways participate in exchange in the absence of *HOT1*.

MATERIALS AND METHODS

Strains and plasmids: Strains containing a *rad1-2*, *rad4*, *rad50-1* or *rad52-1* mutation are congenic isolates kindly provided by R. E. MALONE. These mutant strains were crossed to RLK1-3C (*MATα his4-260 ura3-52 ade2-1 can*^{r}). Following sporulation, tetrads were dissected by standard techniques (SHERMAN, FINK and HICKS 1986). From each cross a Rad$^-$ segregant containing *MAT***a**, *his4-260* and *ura3-52* was obtained. Two further backcrosses to RLK1-3C were conducted for each *rad* mutation. From the third backcross, three Rad$^-$ and three Rad$^+$ progeny were picked for further study. Plasmids pL524 (lacking *HOT1*), and pL623 (containing a 570-bp subclone of *HOT1* called EI, termed EI → *HOT1*) were used to create recombination substrates (Figure 1, A and B, respectively) as described previously (VOELKEL-MEIMAN, KEIL and ROEDER 1987).

To further characterize the effects of *rad1* and *rad52* mutations on *HOT1*-stimulated recombination, the strain RLK88-3C (*MAT***a** *leu2-3,112 his4-260 ura3-52 ade2-1 trp1-HIII lys2-ΔBX can*^{r}) was used. The *trp1-HIII* and *lys2-ΔBX* mutations have been described previously (YUAN and KEIL 1990). Plasmids lacking *HOT1* (pL524), containing EI → *HOT1* (pL623), containing *HOT1* on a 4.6-kb *Bgl*II-B fragment of rDNA, termed *Bgl*II-B *HOT1* (pL559), and containing the inactive orientation of the 570-bp subclone of *HOT1* (pL625, called IE ← *HOT1*) were introduced by transformation into this strain to create recombination substrates. Three independent transformants containing each plasmid properly integrated (Figure 1, A, B, C, and D, respectively) were identified (VOELKEL-MEIMAN, KEIL and ROEDER 1987). A deletion-disruption mutation of the *RAD1* gene, *rad1Δ::LEU2* (called *rad1Δ*), was constructed by deleting a 2.1-kb *Stu*I-*Cla*I-*Cla*I fragment of *RAD1*, filling the 3′ recessed end by the activity of the Klenow fragment of *Escherichia coli* DNA polymerase I and inserting the *LEU2* gene on a 3.2-kb *Bgl*II fragment that was made blunt ended with Klenow fragment. The one-step gene disruption technique (ROTHSTEIN 1983) was used to insert the *rad1Δ* mutation into appropriate strains. The *rad52-8::TRP1* (called *rad52-8*) mutation (obtained from D. SCHILD) was inserted into strains by the same procedure. Southern analysis and phenotypic characterization were used to confirm that proper disruptions had occurred.

The *E. coli* strain MC1066 [*leuB trpC pyrF::*Tn*5* (Kan^R) *araT lacX74 del strA hsdR hsdM*) obtained from M. CASADABAN was used throughout this work.

DNA manipulations: Restriction endonucleases and DNA modification enzymes were purchased from several sources and used according to the manufacturer's specifications. DNA manipulations were performed as described previously (KEIL and ROEDER 1984).

Media and growth conditions: Yeast synthetic complete (SC) and sporulation media were as described by MALONE, GOLIN and ESPOSITO (1980) except that for SC medium L-aspartic acid (74 mg/liter) and valine (140 mg/liter) were added and the pH was not adjusted. Drop-out media (*e.g.*, SC-his) were prepared by omitting the appropriate constituent from SC medium. To select Ura$^-$ cells, 0.8 g of 5-fluoro-orotic acid (5FOA) per liter was added to SC medium. The 5FOA was either synthesized (ALAM, SHIRES and ABOUL-ENEIN 1975) or purchased from PCR Incorporated. Other yeast media were prepared as described in SHERMAN, FINK and HICKS (1986). Bacterial media were formulated as described in MANIATIS, FRITSCH and SAMBROOK (1982). Strains were sporulated by growing on YPD plates overnight at 30°, replicating to sporulation medium and incubating at 30°.

Fluctuation tests, independent recombinants, and data analysis: Fluctuation tests were conducted as described previously (KEIL and ROEDER 1984) except that cultures were grown in SC medium. The number of Ura$^-$ and His$^+$ recombinants were determined by plating appropriate dilutions of the cultures on SC + 5FOA and SC-his, respectively. The recombination frequency was determined by the method of the median (LEA and COULSON 1949). Rates were determined by dividing the frequency by $(\ln N - \ln N_0)$ where N is the final number of cells in the culture and N_0 is the initial number (DRAKE 1970). The median test as described by SIEGEL (1956) was used to test for significant differences between median recombination rates.

Independent His$^+$ or Ura$^-$ recombinants were obtained

by replicating SC plates containing approximately 100 colonies to SC-his and SC + 5FOA. Recombinants from each colony were streaked on SC and a single colony from this streak was tested for appropriate phenotypes. To test for significant differences between the proportion of different types of recombinants produced by the various genotypes, the 2 × 2 contingency test was used (SNEDECOR and COCHRAN 1967).

RESULTS

Assay for the recombination-stimulatory activity of *HOT1*: When inserted at novel locations in the yeast genome, *HOT1* stimulates mitotic recombination in adjacent sequences (KEIL and ROEDER 1984). Two versions of *HOT1* were used in these experiments. One is the 4.6-kbp *Bgl*II-B fragment of rDNA, termed *Bgl*II-B *HOT1*, that contains the 3′ half of the 25S rRNA gene, the nontranscribed spacer, the 5S rRNA gene, and the 5′ sequences encoding the 35S pre-rRNA. The other version, EI → *HOT1* (or IE ← *HOT1* when inserted in the inactive orientation), is a 570-bp subclone derived from the *Bgl*II-B fragment that produces a larger stimulation of recombination than the *Bgl*II-B fragment. This version contains the enhancer and initiation site for the 35S rRNA transcript, and the arrow indicates the direction of RNA polymerase I transcription. These *HOT1*-containing fragments are inserted into a plasmid that can be targeted to integrate at the *his4* locus on chromosome *III*. Integration of these plasmids produces a duplication of *his4* alleles separated by pBR322 and *URA3* sequences (Figure 1). Recombinants that are His^+ or Ura^- can be produced from these duplications. Ura^- recombinants, which can be either His^+ or His^- are defined as excisive recombinants (Figure 2). These can result from a number of different events including simple intrachromatid reciprocal exchange, intrachromatid gene conversion associated with a reciprocal cross-over, unequal-sister-chromatid exchange, or unequal-sister-chromatid conversion. His^+Ura^+ recombinants are produced by events termed gene replacements. These recombinants contain both copies of the repeated chromosome *III* sequences, and the plasmid and *URA3* sequences. Gene replacements can be produced by events including intrachromatid or sister-chromatid gene conversion unassociated with reciprocal exchange or double reciprocal exchange.

Mutations in *RAD1* and *RAD52* decrease the recombination-stimulatory activity of *HOT1*: Mutations in two different genes involved in error-free excision repair (reviewed by HAYNES and KUNZ 1981) were tested for their effect on *HOT1*-stimulated recombination. As shown in Table 1, the *rad1–2* mutation significantly decreases the recombination-stimulatory activity of EI → *HOT1*. However, as shown by the results for strains lacking *HOT1*, *rad1–2* does not significantly alter the rate of recombination in the absence of *HOT1*. *Rad4* does not affect intrachromosomal recombination in the presence or absence of *HOT1*.

Mutations in both *RAD50* and *RAD52*, members of the double-strand-break repair group, were also tested. *Rad52–1* decreases the rate of both His^+ and Ura^- recombination in the presence or absence of *HOT1*. The *rad50–1* mutation does not affect recombination in the presence or absence of *HOT1*.

The strains used in the above studies were derived by three backcrosses. However, there is still considerable variation in the level of recombination in the strains. For example, there is about a sevenfold difference in the rate of His^+ recombination in the *RAD50* and *RAD52* strains containing EI → *HOT1*. To further study the effects of *rad1* and *rad52* on the recombination-stimulatory activity of *HOT1* we constructed isogenic strains containing these mutations by yeast transformation.

The data in Table 2 show that the deletion-disruption mutation *rad1*Δ significantly decreases the rate of *HOT1*-stimulated recombination in almost all cases. This decrease is approximately two- to fivefold for both His^+ and Ura^- recombination. In strains without *HOT1* at *his4* or strains containing the inactive orientation, IE ← *HOT1*, the *rad1*Δ mutation does not affect the rate of recombination. In strains containing the *rad52-8* mutation there is a significant decrease in the rate of both His^+ and Ura^- recombination in the presence or absence of *HOT1*.

Mutations in *RAD1* and *RAD 52* interact synergistically to reduce recombination: The data from the single mutant strains suggest that *rad1* specifically affects *HOT1*-stimulated recombination while *rad52* has a general effect on mitotic intrachromosomal recombination at *HIS4*. To examine the interaction of these mutations, we constructed strains containing both *rad1*Δ and *rad52-8*. As shown in Table 2, the rate of both His^+ and Ura^- recombination decreases significantly in the double mutant strains as compared to the single mutant strains. The only case in which this does not occur is the production of Ura^- cells in the absence of *HOT1*. The synergistic interaction of *rad1* with *rad52* in the presence or absence of *HOT1*, indicates that the effect of *rad1* on recombination is not specific to *HOT1* activity.

Further characterization of recombinants: To further examine recombination in these strains, independent recombinants were isolated from strains that lack *HOT1* and strains containing EI → *HOT1*. Different types of events can lead to the production of His^+ recombinants. Recombination between the repeats can lead to the production of His^+Ura^+ gene replacements and Ura^- excisive recombinants that are either His^+ or His^- (Figure 2). Other events can also lead to the production of cells with these phenotypes. South-

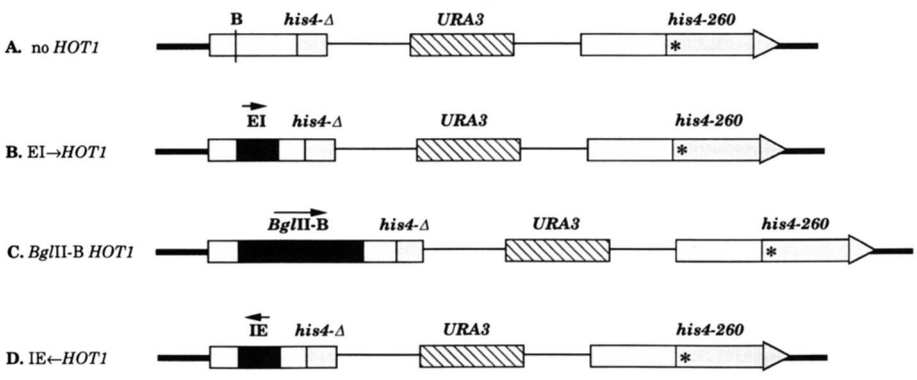

FIGURE 1.—Recombination substrates. Duplications of two different *his4* mutations separated by pBR322 and *URA3* sequences were constructed as described previously (VOELKEL-MEIMAN, KEIL and ROEDER 1987). One of the duplications (A) lacks *HOT1*. The duplication in (B) contains a 570-bp subclone of *HOT1* oriented (EI→) such that it will stimulate exchange between the repeated *his4* sequences. The duplication in (C) contains *HOT1* in the 4.6-kbp *Bgl*II-B fragment of rDNA. The duplication in (D) contains the 570-bp subclone of *HOT1* in the inactive orientation (IE←) that does not stimulate recombination of the repeated sequences. Heavy line: chromosome *III* sequences near *his4*; open block: sequences 5′ of *his4* that are duplicated; dotted block: *his4* gene with the arrowhead representing the 3′ end; thin line: pBR322 sequences; diagonally striped block: *URA3* gene; filled block: subclones of rDNA that contain *HOT1*; star: indicates the relative position of the *his4-260* mutation; the arrow above the *HOT1* fragment indicates the active (→) or inactive (←) orientation for increasing the frequency of His^+ and Ura^- recombinants; B: the *Bam*HI linker at which *HOT1* was inserted.

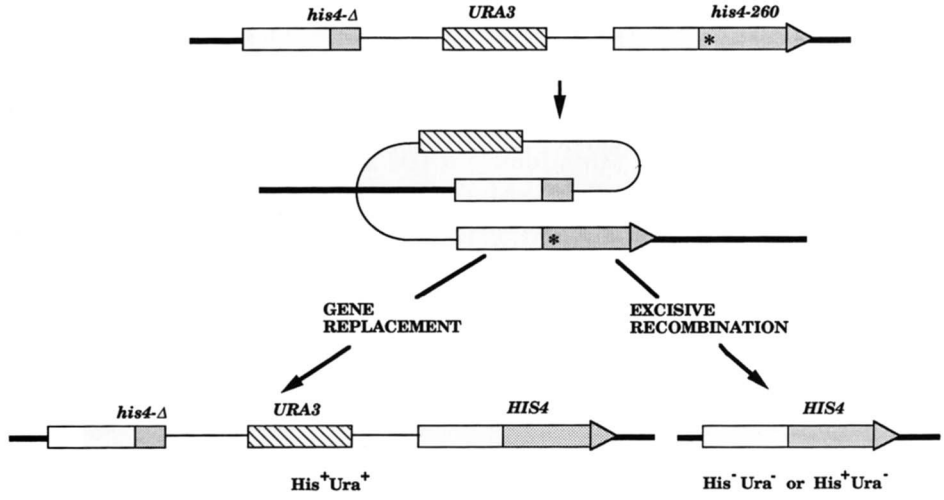

FIGURE 2.—Recombination events. One possible pairing arrangement is diagrammed. Recombination producing a His^+ Ura^+ cell that contains the duplicated *his4* sequences flanking the plasmid and URA3 sequences is defined as gene replacement. Excisive recombinants are Ura^- and have lost one of the *his4* repeats as well as the plasmid and *URA3* sequences. The symbols are described in the legend to Figure 1.

ern analysis was performed to determine whether the chromosomal configuration of independent His^+Ura^+ and Ura^- recombinants was consistent with gene replacement and excisive events, respectively. We define gene replacements as His^+Ura^+ cells that contain the repeated chromosome *III* sequences and the plasmid and *URA3* sequences on a single chromosome (Figure 2). The His^+Ura^+ recombinants from the wild-type and *rad1*Δ strains appear to have resulted from gene replacement (Table 3). However, regardless of the presence or absence of *HOT1*, Southern analysis showed that all 20 His^+Ura^+ recombinants examined from *rad52-8* strains contain two copies of chromosome *III* (Table 3). One of the copies of chromosome *III* contains the parental configuration of the repeated *his4* alleles separated by plasmid and *URA3* sequences, while the other copy of chromosome *III* has undergone excisive recombination. To determine whether these recombinants were disomic ($n + 1$) or *MAT***a**/*MAT***a** diploids, they were crossed to a haploid and a *MATα*/*MATα* diploid. Four His^+Ura^+ recombinants from the *rad52-8* strains lacking *HOT1* and three such recombinants from the *rad52-8* strains containing *HOT1* were tested. All seven recombinants gave fewer than 10% viable spores when crossed to a haploid but more than 70% viable spores when crossed to a *MATα*/*MATα* diploid. Furthermore, in the tetraploids resulting from the latter cross, markers such as *ADE2* segregated $2^+:2^-$, $3^+:1^-$, and $4^+:0^-$ as expected from the genotype of the tetraploid (*ADE2*/*ADE2*/*ade2*/

TABLE 1

Effect of repair and recombination mutants on the rate of *HOT1*-stimulated recombination

Strain	No *HOT1* rate (×10⁵)		EI → *HOT1* rate (×10⁵)	
	His⁺	Ura⁻	His⁺	Ura⁻
rad1-2	1.07 (9)	0.60 (9)	4.63 (18)**	22.4 (9)**
RAD1	1.21 (9)	0.74 (9)	53.6 (18)	196 (9)
rad4	1.34 (9)	0.88 (9)	53.1 (9)	254 (9)
RAD4	1.38 (9)		37.5 (9)	
rad50-1	0.98 (15)	0.53 (15)	42.4 (17)	178 (17)
RAD50	1.69 (18)		35.0 (18)	
rad52-1	0.69 (9)**	0.41 (9)**	0.26 (9)**	2.74 (9)**
RAD52	3.80 (9)	1.26 (9)	235 (9)	238 (9)

The rate of Ura⁻ recombination for *rad4* and *rad50-1* was compared to be combined median rate of Ura⁻ recombination for the 18 *RAD1* and *RAD52* cultures tested. For all other comparisons the recombination rates for the mutant strain were compared to the rates for the appropriate wild-type strain. The number of cultures tested for each strain is shown in parentheses.

* Significantly different at $P = 0.05$; ** significantly different at $P = 0.01$.

TABLE 2

Effect of *rad1Δ* and *rad52-8* on the rate of *HOT1*-stimulated recombination

Strain	No *HOT1* rate (×10⁵)		*Bgl*II-B *HOT1* rate (×10⁵)		EI → *HOT1* rate (×10⁵)		*IE* ← *HOT1* rate (×10⁵)	
	His⁺	Ura⁻	His⁺	Ura⁻	His⁺	Ura⁻	His⁺	Ura⁻
Rad⁺	2.00	1.63	6.27	7.72	90.1	360	1.48	2.81
rad1Δ	1.42	1.64	2.68**	4.80	18.5**	120**	1.12	2.19
rad52-8	0.30**	0.65*	0.21**	1.82**	0.35**	3.97**	0.10**	1.03*
rad1Δ, rad52-8	0.017**	0.26	0.023**	0.18**	0.10*	0.55**	0.01*	0.13*

The rate of recombination was determined by assaying 27 cultures for the wild-type strains, 18 cultures for the *rad1Δ* and *rad52-8* strains and 9 cultures for the *rad1Δ rad52-8* strains. Statistical significance of the data for the *rad1Δ* and *rad52-8* strains is indicated for comparisons of the recombination rates for these mutant strains to the wild-type strains. Significance for comparisons of the data for the *rad1Δ rad52-8* strains to the *rad52-8* strains is shown. The symbols are as described for Table 1.

ade2). Thus, these cells are diploids apparently derived by endomitosis and excisive recombination and will be called His⁺Ura⁺ diploids. In crosses involving these His⁺Ura⁺ diploids, the His⁺ and Ura⁺ phenotypes often segregated to different spores showing that the *HIS4* and *URA3* genes are in repulsion.

In the *rad1Δ rad52-8* strains only a few (4 of 20) of the His⁺Ura⁺ recombinants were diploid. In the absence of *HOT1*, 3 of 12 His⁺Ura⁺ recombinants were diploids, while one of eight such recombinants from the strains with *HOT1* was a diploid (Table 3). Genetic analysis of two of these recombinants showed that the *HIS4* and *URA3* genes were in repulsion. The haploid His⁺Ura⁺ recombinants in the *rad1Δ rad52-8* strains do not appear to result from spontaneous mutation. In *rad1Δ rad52-8* strains the rate of spontaneous reversion of *his4-260* to His⁺ (0.8×10^{-8} in the absence of *HOT1* and 2.8×10^{-8} when EI → *HOT1* is present) is more than ninefold lower than the rate of His⁺Ura⁺ recombination (see below and Table 5). Thus most of the haploid His⁺Ura⁺ cells in the *rad1Δ rad52-8* strains result from recombination rather than mutation.

To investigate whether the production of these diploid His⁺Ura⁺ recombinants is an allele-specific phenotype of the *rad52-8* disruption mutation, His⁺Ura⁺ recombinants derived from *rad52-1* strains were tested. Southern analysis showed that two of four such recombinants from a strain lacking *HOT1* and one of four recombinants from a strain containing EI → *HOT1* contained two copies of chromosome *III*. The *rad52-8* and the *rad52-1* strains used were not isogenic. Therefore, this difference may be due to a difference between the *rad52-8* and *rad52-1* alleles or to other genetic differences between the strains used.

His⁺ excisive recombinants are Ura⁻ and should have lost one copy of the *his4* repeat as well as the plasmid and *URA3* sequences. Southern analysis showed this had occurred for all of the strains. Tetrads derived from crossing His⁺Ura⁻ recombinants from the *rad52-8* and *rad1Δ rad52-8* strains to a haploid gave greater than 80% spore viability, and the chromosome *III* markers segregated 2:2 showing that the recombinants were not diploid or disomic.

Ura⁻ recombinants can be either His⁺ or His⁻. In both cases excisive recombinants are expected to lose one of the *his4* repeats and the plasmid and *URA3*

TABLE 3

Summary of molecular and genetic characterization of events

Strain	Number of independent recombinants				
	His^+Ura^+			Ura^-His^-	
	Gene replacements	Diploids	Ura^-His^+ Excisive	Excisive	Mutations
No *HOT1*					
Rad^+	17	0	8	4	0
rad1Δ	12	0	7	3	0
rad52-8	0	14	8	4	0
rad1Δ rad52-8	9	3	7	6	8
EI → *HOT1*					
Rad^+	8	0	6	4	0
rad1Δ	8	0	8	3	0
rad52-8	0	6	8	4	0
rad1Δ rad52-8	7	1	5	6	7

Independent His^+ and Ura^- colonies were characterized by Southern analysis and genetic techniques as described in the text to classify the event that produced the putative recombinant.

sequences. All of the independent Ura^- recombinants from wild-type, *rad1Δ*, and *rad52-8* strains as well as the Ura^-His^+ recombinants from the *rad1Δ rad52-8* strains showed the pattern expected for excisive recombinants. From the *rad1Δ rad52-8* strains, however, about 55% of the independent Ura^-His^- recombinants still contained the parental configuration of the duplication at *his4*. Two lines of evidence indicate that these cells result from spontaneous mutations in the *URA3* gene on chromosome *III*. First, Southern blot analysis shows that the restriction maps of the recombinant and parental chromosomes are identical, indicating they were not derived from ectopic recombination with the *ura3–52* mutation on chromosome *V*, which contains a Ty element (ROSE and WINSTON 1984). These results also show that these Ura^- cells result from point mutations or other small mutations. Second, these strains fail to complement a *ura3* strain but they do complement a strain containing a *ura1* mutation. As expected, these Ura^-His^- recombinants still give rise to His^+ recombinants at the rate observed for *rad1Δ rad52-8* strains.

The proportions of different types of recombinants are altered by *HOT1*, *rad1* and *rad52*: Independent His^+ recombinants were isolated and tested to determine if they were Ura^+ or Ura^-. The results from this analysis are shown in Table 4A. For the wild-type strains there is no difference in the proportion of His^+Ura^+ recombinants in the absence of *HOT1* and when *Bgl*II-B *HOT1* is present. However, when EI → *HOT1* or IE ← *HOT1* is present there is a significant decrease in the proportion of His^+Ura^+ recombinants. Thus, when IE ← *HOT1* is present it does not affect the rate of His^+ recombination (Table 2) but it does significantly alter the proportions of the different events.

As compared to wild type, the *rad1Δ* strains show an increase in the proportion of independent His^+ recombinants that are Ura^+. Thus the *rad1Δ* mutation decreases the proportion of His^+ recombinants that are formed by excisive recombination in the presence or absence of *HOT1*. This finding further shows that *rad1Δ* affects recombination in the presence or absence of *HOT1*.

The results from the *rad52-8* strains generally show a decrease in the proportion of His^+ recombinants that are Ura^+ as compared to wild type. This is not true for the strains containing EI → *HOT1*, which have a very low proportion of His^+Ura^+ recombinants even in the wild-type strains. However, as described above, these His^+Ura^+ recombinants from *rad52-8* strains are not derived by gene conversion. As shown by the numbers in parentheses, all of the His^+Ura^+ recombinants tested in *rad52-8* strains containing EI → *HOT1* or lacking *HOT1* are excisive recombinant diploids. We assume this is also true for the *rad52-8* strains containing *Bgl*II-B *HOT1* or *HOT1*.

In the *rad1Δ rad52-8* strains the proportion of His^+ recombinants that are Ura^+ is approximately 50% in the presence or absence of *HOT1*. As shown in Table 4A, this proportion is in general different from that found in strains containing either single mutation. The proportion of these recombinants that are diploids rather than gene replacements is shown in parentheses.

Ura^- excisive recombinants can be either His^+ or His^-. As shown by the data in Table 4B the proportion of the Ura^- recombinants that are His^- in Rad^+ strains is altered by the presence of *HOT1* in the active orientation either in the *Bgl*II-B fragment or in EI → *HOT1*. In the *rad1Δ* strains this proportion is changed only in the presence of *Bgl*II-B *HOT1*. In the *rad52-8* strains the proportion of Ura^- recombinants that are His^- is higher than the proportion in wild-type strains

TABLE 4

Effect of *rad1Δ* and *rad52-8* on the types of recombinants formed

	Rad$^+$	*rad1Δ*	*rad52-8*	*rad1Δ rad52-8*
A. Percent of independent His$^+$ recombinants that are Ura$^+$				
No *HOT1*	54.2	75.3**	24.1** (100%)	51.5**, ** (25%)
*Bgl*II-B *HOT1*	48.6	94.7**	16.1** (ND)	41.3**, ** (ND)
EI → *HOT1*	10.0**	44.7**	14.1 (100%)	55.4^{NSD}, ** (12.5%)
IE ← *HOT1*	31.9**	76.0**	14.3** (ND)	58.9*, ** (ND)
B. Percent of independent Ura$^-$ recombinants that are His$^-$				
No *HOT1*	78.7	85.6	88.0	92.7[a]
*Bgl*II-B *HOT1*	60.7**	90.0**	97.2**	89.5[a]
EI → *HOT1*	88.3*	92.5	97.7**	96.0[a]
IE ← *HOT1*	87.9	94.0	96.2*	94.1[a]

More than 50 independent recombinants were scored for each genotype. Statistical significance for the various Rad$^+$ strains is indicated for comparisons of the *HOT1*-containing strains to the strain lacking *HOT1*. For the strains containing *rad1Δ* or *rad52-8* statistical significance is shown for the comparison to the appropriate wild-type strain. The data for the *rad1Δ rad52-8* strains were compared to the data for *rad1Δ* and *rad52-8* strains, respectively. The numbers in parentheses show the percent of His$^+$Ura$^+$ recombinants that were diploid.

[a] These numbers were corrected for the 55.6% (15 or 27) of the Ura$^-$His$^-$ colonies that arose from spontaneous mutation as determined by Southern analysis. These events occur in the presence or absence of *HOT1*. Therefore, corrections were made for all of the strains even though only strains lacking *HOT1* and strains containing EI → *HOT1* were actually tested.

ND = not determined; NSD = not significantly different; other symbols are as described for Table 1.

TABLE 5

Calculated rates of events

	Rate of His$^+$Ura$^+$ (×10^5)		Rate of His$^+$Ura$^-$ (×10^5) from		Rate of Ura$^-$His$^-$ (×10^5)	
Strain	Gene replacement	Diploids	Indep His$^+$	Indep Ura$^-$	Recombination	Mutation
No *HOT1*						
Rad$^+$	1.08	<0.06	0.92	0.35	1.28	<0.32
rad1Δ	1.07	<0.09	0.35	0.24	1.40	<0.46
rad52-8	<0.005	0.07	0.23	0.08	0.57	<0.14
rad1Δ rad52-8	0.007	0.002	0.008	0.01	0.11	0.14
EI → *HOT1*						
Rad$^+$	9.01	<1.1	81.1	42	318	<79
rad1Δ	8.27	<1.0	10.23	9.0	111	<37
rad52-8	<0.01	0.05	0.30	0.09	3.88	<0.96
rad1Δ rad52-8	0.05	0.01	0.04	0.01	0.25	0.29

Rates were calculated by multiplying the rate of His$^+$ or Ura$^-$ recombination (Table 2) by the proportion of the various events as determined by molecular and genetic analysis (Tables 3 and 4). Where no events were detected, an upper limit on the rate was determined by assuming that one of the cells examined by Southern blot analysis (Table 3) was of the indicated type.

whenever an active or inactive form of *HOT1* is present. Strains containing *rad1Δ rad52-8* have a very high proportion of Ura$^-$ recombinants that are His$^-$ even after correcting for the Ura$^-$ cells produced by spontaneous mutation.

The frequency of gene replacement is not affected in *rad1* strains: The rate of various types of recombination can be calculated by multiplying the proportions of different events from Tables 3 and 4 by the rates of His$^+$ and Ura$^-$ recombination in Table 2. The results of these calculations are shown in Table 5. Most interestingly, the rate of excisive events, both His$^+$Ura$^-$ and His$^-$Ura$^-$, is decreased by *rad1Δ* in strains containing *HOT1*. However, the rate of gene replacement events in *rad1Δ* strains containing *HOT1* is not decreased. In strains lacking *HOT1*, *rad1Δ* does not have a dramatic effect on any one category of recombination event. In the presence or absence of *HOT1*, recombinants consistent with a gene replacement event were not detected in *rad52-8* strains, and the rate of excisive recombination is decreased in these strains compared to wild type. For excisive events there is a synergistic interaction between *rad1Δ* and *rad52-8* in strains containing or lacking *HOT1*. However, this is not true for the production of His$^+$Ura$^+$ gene replacements. This is most clearly seen for strains containing *HOT1*. The rate of formation of His$^+$Ura$^+$ cells in the *rad52-8* strains is 0.05×10^{-5}. All six recombinants analyzed by Southern blots were diploids, so the rate of gene replacement is less than 0.01×10^{-5} (the rate of gene replacement if 1 of the 6 had been derived from such an event). However, the rate of gene replacement for the double-mutant strains is 0.05×10^{-5}, which is higher than the rate for the *rad52-8* strains.

The rate of His$^+$Ura$^-$ recombination can be calcu-

TABLE 6

Fate of *HOT1* or *Bam*HI linker in recombinants

Strain	Independent recombinants						
	His⁺Ura⁺			His⁺Ura⁻		His⁻Ura⁻	
	1 copy[a]	2 copies[a]	Multiple[b]	+[c]	−[c]	+[c]	−[c]
Rad⁺							
No *HOT1*	10	7	0	7	1	2	2
EI → *HOT1*	6	2	0	6	0	1	3
rad1Δ							
No *HOT1*	7	3	2	7	0	0	3
EI → *HOT1*	7	1	0	8	0	0	3
rad52-8							
No *HOT1*	0	0	0	8	0	1	3
EI → *HOT1*	0	0	0	8	0	1	3
rad1Δ rad52-8							
No *HOT1*	8	1	0	7	0	3	3
EI → *HOT1*	6	1	0	5	0	1	5

Independent His⁺ and Ura⁻ recombinants were characterized by Southern analysis. Diploid His⁺ Ura⁺ recombinants and spontaneous Ura⁻ mutants were excluded from this analysis.

[a] Number of copies of *Bam*HI linker for non-*HOT1* strains or *HOT1* for strains containing EI → *HOT1*. The *Bam*HI linker is the site at which *HOT1* is inserted (Figure 1A). In all cases where there is one copy of the *Bam*HI linker or *HOT1* it is in the same repeat as in the parental strain.

[b] There is at least a triplication of the *his4* repeated sequences.

[c] + indicates the presence of either the *Bam*HI linker or EI → *HOT1* as appropriate for a given strain. − indicates the absence of these elements in the recombinants.

lated from either the His⁺ data or the Ura⁻ data. These two rates should be equal. However, they are not (Table 5). In general the rate calculated from the Ura⁻ data is two- to sixfold lower than the rate determined from the His⁺ data. This may result from the phenotypic lag associated with growth of *ura3* cells on medium containing 5FOA (RONNE and ROTHSTEIN 1988; YUAN and KEIL 1990).

Southern analysis of independent recombinants: Further information regarding the recombination events can be obtained from Southern analysis of DNA derived from independent recombinants. The repeated *his4* genes differ by the *his4–260* mutation. In addition, strains lacking *HOT1* contain a *Bam*HI linker inserted at the *Pvu*II site 651 bp 5′ of the *HIS4* coding sequence. This is the site at which *HOT1* is inserted. It is possible to follow the fate of the *Bam*HI linker or *HOT1* in recombinants. Independent recombinants from strains lacking *HOT1* or containing EI → *HOT1* were analyzed and the results are presented in Table 6. Examination of the His⁺Ura⁺ gene replacements shows that three types of events are observed. The majority of these recombinants have the parental configuration of *Bam*HI or *HOT1*. A second type of recombinant has *Bam*HI or *HOT1* present in both of the *his4* repeats. We found this type of recombinant in all of the strains that gave rise to gene replacements. The final type of gene replacement had at least three copies of the repeated *his4* sequence. These may have been produced by a gene conversion followed by an unequal-sister-chromatid exchange. Two of these were found in the *rad1Δ* strain. Only a relatively small number of recombinants can be readily examined by these techniques. Larger numbers of recombinants would have to be tested to determine if there is a significant difference in the proportion of the types of recombinants recovered from the different genotypes.

His⁺Ura⁻ recombinants can be derived either as independent His⁺ or Ura⁻ cells. Examining both cases we find that almost all of them (56 of 57) contain the *Bam*HI linker or *HOT1*. This is consistent with a reciprocal exchange occurring 3′ of the *his4–260* mutation to produce a His⁺ recombinant. The majority of independent Ura⁻His⁻ recombinants lack the *Bam*HI linker or *HOT1*. In *rad1Δ* strains this is the only class of Ura⁻His⁻ recombinants observed. A reciprocal exchange upstream of the *Bam*HI linker (or *HOT1*) would produce a Ura⁻His⁻ recombinant lacking the linker (or *HOT1*). The Ura⁻His⁻ recombinants that contain the *Bam*HI linker (or *HOT1*) could be produced by a reciprocal exchange that occurred between the *his4–260* mutation and the *Bam*HI linker (or *HOT1*).

DISCUSSION

We used intrachromosomal recombination between direct repeats to further characterize the recombination-stimulatory activity of *HOT1*. Some, but not all, of the genes in the error-free excision repair and double-strand-break repair pathways affect *HOT1* activity. Our results show that a mutation in either *rad1*

or *rad52* decreases *HOT1* activity. *HOT1* stimulation of plasmid excision but not gene replacement is decreased in *rad1*Δ strains. *Rad52–8* decreases both *HOT1*-stimulated plasmid excision and gene replacement. In *rad1*Δ *rad52-8* strains, a synergistic interaction leads to very low levels of recombination. *HOT1* stimulates the formation of gene replacements in *rad1*Δ *rad52-8* strains. These results indicate that *HOT1* uses multiple recombination pathways to stimulate recombination.

Effects of *HOT1* on intrachromosomal recombination: In wild-type strains the EI → *HOT1* subclone stimulates both plasmid excision and gene replacement (Table 5). However, the rate of excision is stimulated much more (approximately 200-fold) than the rate of gene replacement (about sixfold). *HOT1*-stimulated recombination may preferentially produce excision events. Alternatively, this finding may result from the recombination substrate used to measure *HOT1* activity. The relative position of *HOT1* and the *his4* alleles may dramatically affect the recombination events that can be recovered and, therefore, influence the level of stimulation measured. NICKOLOFF *et al.* (1989) studied effects of double-strand breaks introduced by the HO endonuclease on direct-repeat recombination. They found that gene replacement could be stimulated anywhere from 10-fold to more than 100-fold depending on the configuration of the double-strand break site and the recombining alleles.

THOMAS and ROTHSTEIN (1989a) found that only plasmid excision was stimulated when high levels of RNA polymerase II transcription occurred. The difference between the results reported here and those of THOMAS and ROTHSTEIN (1989a) may be that RNA polymerase I transcription (from *HOT1*) stimulates recombination by a different mechanism than RNA polymerase II transcription. Alternatively, this difference may result from the use of different recombination substrates.

The other forms of *HOT1*, *Bgl*II-B *HOT1* and IE ← *HOT1*, also affect recombination in this assay system in wild-type strains. As found previously *Bgl*II-B *HOT1* produces a much smaller increase in recombination than EI → *HOT1* (VOELKEL-MEIMAN, KEIL and ROEDER 1987). The IE ← *HOT1* construct that is inactive in stimulating the rate of recombination (Table 5 and VOELKEL-MEIMAN, KEIL and ROEDER 1987) does have a subtle effect on recombination in that a significantly smaller percentage of His^+ recombinants are gene replacements (Table 4). Thus the presence of the IE ← *HOT1* insertion may affect the resolution of recombination intermediates or the types of recombination events that occur although it does not alter the rate of recombination.

Effects of *rad* mutations on the recombination-stimulatory activity of *HOT1*: *Rad1*Δ significantly reduces the ability of *HOT1* to stimulate plasmid excision but does not affect the increased production of gene replacements by *HOT1*. THOMAS and ROTHSTEIN (1989b) saw a similar, but smaller, effect of *rad1* on RNA polymerase II-stimulated recombination. In the absence of *HOT1*, *rad1*Δ does not affect the rate of recombination but it does significantly increase the proportion of His^+ recombinants that are gene replacements (Table 4). This is also true for strains containing the inactive, IE ← *HOT1*, orientation of *HOT1*. SCHIESTL and PRAKASH (1988) and KLEIN (1988) found that the rate of mitotic excisive recombination for nontandem duplications is decreased in *rad1* strains. KLEIN (1988) found that the rate of gene replacement is not affected by *rad1*.

We find that both in the presence and absence of *HOT1*, *rad52-8* abolishes gene replacement events and also decreases the rate of excision (Table 5). Using duplications at the *his4* locus, JACKSON and FINK (1981) found that *rad52* preferentially affected the frequency of gene replacement while not affecting the occurrence of excisive events. KLEIN (1988) and NICKOLOFF *et al.* (1989) found that excisive recombination was decreased in *rad52* strains. The differences between these results may be due to the different duplications studied or to variation in the background genotype of the strains used.

Together *rad1*Δ and *rad52-8* interact synergistically to decrease recombination in the presence or absence of *HOT1*. This indicates that these two genes function in different recombination pathways that can compete for at least some of the same intermediates. A similar observation has been made by SCHIESTL and PRAKASH (1988), KLEIN (1988) and THOMAS and ROTHSTEIN (1989b). *HOT1* stimulates the rate of gene replacement but not plasmid excision in *rad1*Δ *rad52-8* strains. Thus a portion of the activity of *HOT1* occurs by a *RAD1*- and *RAD52*-independent pathway.

Mutations in *RAD4* and *RAD50* affect interchromosomal mitotic recombination (MONTELONE, HOEKSTRA and MALONE 1988; MALONE and ESPOSITO 1981; MALONE 1983). In our intrachromosomal assay, regardless of the presence or absence of *HOT1*, these mutations did not alter the rate of recombination. In light of the synergistic interaction of *rad1* and *rad52*, studies examining the interaction of these *rad* mutations with *rad1* and *rad52* are warranted. However, it is clear that interchromosomal and intrachromosomal exchange are affected differently by some mutations involved in the repair and recombination of DNA. For example, *rad50* increases spontaneous mitotic interchromosomal recombination three- to tenfold (MALONE and ESPOSITO 1981; MALONE 1983) but, as shown in Table 1, does not affect intrachromosomal exchange.

His^+ Ura^+ diploids in *rad52-8* strains: The most

striking observation in *rad52-8* strains is that His^+Ura^+ recombinants are not products of gene replacement but rather are diploids apparently derived by endomitosis and excisive recombination. This occurs in the presence or absence of *HOT1*. There are several possible explanations for this observation. One is that recombination and endomitosis are associated events in these cells. Possibly recombination is initiated but cannot be properly resolved. The cells may undergo an abortive mitosis without nuclear division, followed by resolution of the recombination intermediate. An alternative explanation is that in *rad52-8* strains endomitosis results in the production of diploid cells that then can undergo intrachromosomal recombination on one chromosome. This results in the production of a diploid cell with one His^+Ura^- chromosome and one His^-Ura^+ chromosome. The rate of recombination to produce a His^+Ura^- chromosome in a *rad52-8* strain in the absence of *HOT1* is 0.23×10^{-5} (Table 5). If preexisting diploids in the *rad52-8* culture produce the His^+Ura^+ diploids, one-seventh of the cells in the culture would have to be diploid to produce the observed His^+Ura^+ diploid rate of 0.07×10^{-5} [that is $(0.07 \times 10^{-5})/(2 \times (0.23 \times 10^{-5}))$]. Experiments are currently in progress to determine the frequency of diploid cells present in cultures of *rad52-8* strains.

Other examples of genomic instability have been reported in *rad52* cells. MORTIMER, CONTOPOULOU and SCHILD (1981) showed that there is a high frequency of chromosome loss during mitosis in diploids homozygous for *rad52*. HABER and HEARN (1985) found that mitotic interchromosomal gene conversion in *rad52* strains often is accompanied by chromosome loss. THOMAS and ROTHSTEIN (1989b) recovered cells that were disomic for the chromosome containing their recombination substrate among recombinants from *rad52-8* strains. L.-W. YUAN and R. L. KEIL (unpublished results) have observed both diploid and disomic recombinants in another *rad52-8* strain.

Mechanism of recombination stimulation by *HOT1*: The recombination-stimulatory activity of *HOT1* appears to depend on the ability of this sequence to initiate high levels of transcription (VOELKEL-MEIMAN, KEIL and ROEDER 1987; STEWART and ROEDER 1989). This transcription may permit the introduction of lesions in the DNA that stimulate recombination. Possible lesions include single-stranded regions of DNA, nicks, breaks or RNA:DNA hybrids as suggested by ROSENBERG (1988). *RAD52* has been shown to be required for the recombinational repair of double-strand breaks in mating-type switching (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981; STRATHERN *et al.* 1982), yeast transformation (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981), and homologous recombination (NICKOLOFF *et al.* 1989) during mitosis. It has not been shown whether *RAD52* is also required for the repair of other lesions in DNA but RESNICK *et al.* (1984) found that single-strand nicks accumulate in *rad52* cells during meiosis. Our finding that part of the *HOT1* activity is *RAD52*-dependent suggests that some of these lesions may be double-strand breaks, may be processed into double-strand breaks or may be other lesions whose repair requires *RAD52*. Using an interchromosomal recombination assay for *HOT1* activity, VOELKEL-MEIMAN and ROEDER (1990) found that the chromosome containing *HOT1* is preferentially the recipient of information during gene conversion. This is predicted for recombination initiated by double-strand breaks (SZOSTAK *et al.* 1983). Studying the effects of double-strand breaks on mitotic exchange of direct repeats, J. A. NICKOLOFF and F. HEFFRON (personal communication) found that the HO cleavage site is absent in recombinants recovered following HO-nuclease induced recombination. Thus the HO-double-strand break site is always lost during recombination. Since we find that *HOT1* is frequently recovered in recombinants (Table 6), it appears that *HOT1* need not be the site of a double-strand break. Rather, *HOT1* may increase the frequency of double-strand breaks in adjacent DNA, such as the direct repeats or the intervening plasmid sequences. NICKOLOFF *et al.* (1989) found that double-strand breaks in either of these regions stimulated both gene conversion and excisive exchange. THOMAS and ROTHSTEIN (1989a) proposed that RNA polymerase II transcription stimulates recombination by increasing the occurrence of double-strand breaks in the plasmid sequences separating the direct repeats.

RAD1 is required for the incision of UV-damaged DNA (REYNOLDS and FRIEDBURG 1981; WILCOX and PRAKASH 1981). Since UV-excision repair involves the formation and then repair of a single-strand gap, *RAD1* may be involved in the formation or repair of similar lesions during recombination. SCHIESTL and PRAKASH (1988) previously proposed such a role for *RAD1* in recombination. *Rad1*Δ has a significant effect on the rate of excisive recombination when *HOT1* is present but not when it is absent for this direct-repeat recombination assay. Therefore, instead of uniformly increasing the occurrence of all lesions, *HOT1* appears to preferentially increase the proportion of lesions that are processed by the *RAD1*-dependent pathway. The synergistic interaction of *rad1*Δ and *rad52-8* indicates that some lesions can be processed by either recombination pathway.

Instead of allowing more frequent introduction of lesions in DNA, *HOT1* could stimulate recombination by affecting another step in exchange such as pairing, conversion-tract length, or resolution. AHN and LIVINGSTON (1986) found that the average length of

conversion for mitotic recombination is 0.5 kb. If *HOT1* increased the conversion-tract length, more recombination events that initiated at a distance from the *his4–260* mutation would include this site and increase the frequency of His^+ recombinants. However, our data indicate that *HOT1* does not affect conversion-tract length. In wild-type strains, the proportion of His^+ Ura^+ gene convertants in which *HOT1* is coconverted with the wild-type *his4–260* sequence is not significantly different from the proportion of coconversion for the *Bam*HI linker and the wild-type *his4–260* sequence (Table 6). *HOT1* also does not appear to introduce a preferential resolution site for exchange. If, for example, *HOT1* introduced a resolution site that usually produced reciprocal exchange, it could increase the rate of plasmid excision. Such a site would alter the position of exchanges. We find that in wild-type strains EI → *HOT1* only slightly affects the position of exchange in excisive events as judged by the proportion of Ura^- recombinants that are His^- (Table 4). More precise localization of the position of the exchange by examining independent Ura^- recombinants for the presence or absence of *HOT1* or the *Bam*HI linker (in strains lacking *HOT1*) also shows no dramatic difference between strains containing and lacking *HOT1* (Table 6).

Mutations in *RAD52* do not affect rDNA recombination (ZAMB and PETES 1981; PRAKASH and TAILLON-MILLER 1981; R. L. KEIL, Y.-H. LIN and A. D. MCWILLIAMS, unpublished results). Recombination in the rDNA is not decreased in *rad1Δ* or *rad1Δ rad52-8* strains (R. L. KEIL, Y.-H. LIN and A. D. MCWILLIAMS, unpublished results). Why then is *HOT1* activity decreased by these mutations? It is possible that *HOT1* and *RAD1/RAD52* are involved in different parts of the recombination pathway. For example, *HOT1* may affect initiation of exchange while *RAD1* and *RAD52* are involved in a later step such as resolution. When *HOT1* is inserted at *HIS4*, an exchange event may be initiated in *HOT1* and then branch migrate into adjacent non-rDNA sequences. Once the recombination intermediate leaves *HOT1*, the exchange may be dependent on all of the machinery normally required for non-rDNA recombination including *RAD1* and *RAD52*. Thus the recombination-stimulatory activity of *HOT1* would be reduced by mutations in *RAD1* and/or *RAD52*. Alternatively, it is possible that the rDNA, which is contained in the nucleolus, is inaccessible to the gene products required for the *RAD1*- and *RAD52*-dependent recombination pathways. These findings do not mean that *HOT1* does not function in rDNA recombination. There may be other factors that are required for *HOT1* activity both at *his4* and in the rDNA. We have recently isolated mutations that affect both rDNA recombination and the stimulation of recombination by *HOT1* at *his4* (Y.-H. LIN and R. L. KEIL, manuscript in preparation).

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