Mutations Affecting RNA Polymerase I-Stimulated Exchange and rDNA Recombination in Yeast

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ABSTRACT

HOT1 is a *cis*-acting recombination-stimulatory sequence isolated from the rDNA repeat unit of yeast. The ability of *HOT1* to stimulate mitotic exchange appears to depend on its ability to promote high levels of RNA polymerase I transcription. A qualitative colony color sectoring assay was developed to screen for *trans*-acting mutations that alter the activity of *HOT1*. Both hypo-recombination and hyper-recombination mutants were isolated. Genetic analysis of seven *HOT1* recombination *m*utants (*hrm*) that decrease *HOT1* activity shows that they behave as recessive nuclear mutations and belong to five linkage groups. Three of these mutations, *hrm1*, *hrm2*, and *hrm3*, also decrease rDNA exchange but do not alter recombination in the absence of *HOT1*. Another mutation, *hrm4*, decreases *HOT1*-stimulated recombination but does not affect rDNA recombination or exchange in the absence of *HOT1*. Two new alleles of *RAD52* were also isolated using this screen. With regard to *HOT1* activity, *rad52* is epistatic to all four *hrm* mutations indicating that the products of the *HRM* genes and of *RAD52* mediate steps in the same recombination pathway. Finding mutations that decrease both the activity of *HOT1* and exchange in the rDNA supports the hypothesis that *HOT1* plays a role in rDNA recombination.

RECOMBINATION hotspots, DNA sequences that increase the frequency of genetic exchange in adjacent regions, have been identified in prokaryotes (STAHL, CRASEMANN and STAHL 1975) and eukaryotes (ANGEL, AUSTIN and CATCHESIDE 1970; GUTZ 1971; MACDONALD and WHITEHOUSE 1979; KEIL and ROEDER 1984). In yeast, a *cis*-acting recombination hotspot, *HOT1*, stimulates mitotic recombination when inserted at novel locations in the genome (KEIL and ROEDER 1984). *HOT1* is derived from the ribosomal DNA (rDNA) repeat unit. These units are present as a cluster of tandemly arrayed repeats that are homogeneous in sequence (PETES, HEREFORD and SKRYABIN 1978). It has been suggested that *HOT1* may play a role in maintaining sequence identity of the repeats by stimulating recombination among the repeats (KEIL and ROEDER 1984; ROEDER, KEIL and VOELKEL-MEIMAN 1986). To date, however, it has not been shown that *HOT1* plays a role in rDNA recombination. Therefore, the recombination-stimulatory activity of *HOT1* described in this paper refers to the ability of *HOT1* to stimulate exchange outside of rDNA. Recombination involving the rDNA repeats is simply called rDNA exchange.

The mechanism by which *HOT1* stimulates recombination and the function of *HOT1* in rDNA exchange can be studied by identifying *trans*-acting factors that affect these activities. Previously, we found that mutations in either *RAD52* or *RAD1*, genes involved in DNA repair and recombination, decrease *HOT1* activity (ZEHFUS *et al.* 1990). The requirement of *RAD52* for *HOT1* activity suggests that double-strand breaks play a role in this recombination. The role of *RAD1* in mitotic recombination is unclear. Its involvement in UV-excision repair suggests that it may be required for the formation or repair of single-strand gaps. In *rad1 rad52* strains, there is a synergistic effect on the frequency of excision events indicating that the two mutations are involved in different recombination pathways that compete for a common substrate (ZEHFUS *et al.* 1990; THOMAS AND ROTHSTEIN 1989). Recombination in the rDNA is not decreased in *rad52*, *rad1* or *rad1 rad52* strains (ZAMB AND PETES 1981; PRAKASH and TAILLON-MILLER 1981; R. L. KEIL, unpublished results).

Although the mechanism by which *HOT1* stimulates recombination is unknown, there appears to be an association between recombination and high levels of RNA polymerase I (pol I) transcription. Subcloning (VOELKEL-MEIMAN, KEIL AND Roeder 1987) and linker insertion (STEWART and ROEDER 1989) studies showed that the sequences required for the recombination-stimulatory activity of *HOT1* are located in the same regions as the enhancer (ELION and WARNER 1984, 1986) and initiation site (KLEMENZ and GEIDUSCHEK 1980; BAYEV *et al.* 1980) for 35S rRNA transcription. To stimulate recombination the initiation site must be oriented such that transcription initiated

in this element can proceed across the recombining sequences (VOELKEL-MEIMAN, KEIL and ROEDER 1987). In addition, the activity of *HOT1* is eliminated when the RNA polymerase I transcription terminator is inserted between *HOT1* and the recombining sequences (VOELKEL-MEIMAN, KEIL AND ROEDER 1987). Analysis of linker insertion mutations in *HOT1* showed that mutations which decrease its recombination-stimulatory activity also decrease the level of transcription (STEWART and ROEDER 1989). Recombination and transcription appear to be associated in other instances. In yeast mating-type switching, only transcriptionally active copies of the three mating-type loci are cleaved by the HO endonuclease during switching (KLAR, STRATHERN and ABRAHAM 1984). The promoter region of *ARG4* contains an initiation site for meiotic gene conversion (NICOLAS *et al.* 1989). Transcription from the *GAL10* promoter stimulates mitotic recombination between direct duplications of *gal10* genes (THOMAS and ROTHSTEIN 1989). In mammalian cells, rearrangement of immunoglobulin genes is enhanced by transcription (BLACKWELL *et al.* 1986; SCHLISSEL and BALTIMORE 1989).

To further investigate the mechanism by which *HOT1* stimulates exchange, we have developed a colony color sectoring assay (HIETER *et al.* 1985; KOSHLAND, KENT and HARTWELL 1985) to isolate additional *trans*-acting mutations that affect *HOT1* activity. Using this assay, we have identified both hypo-recombination mutants and hyper-recombination mutants. The mutants that decrease recombination identify five genes, one of which is *RAD52*. The other four hypo-recombination mutations affect exchange when *HOT1* is present but not when it is absent. Three of these mutations affect rDNA recombination suggesting that *HOT1* plays a role in rDNA exchange.

MATERIALS AND METHODS

Strains: The yeast strains used in this study are described in Table 1. *Escherichia coli* strain MC1066 (*leuB trpC pryF::Tn5* (Kan^R) *araT lacX74 del strA hsdR hsdM*), obtained from M. CASADABAN, was used throughout these studies.

Plasmid constructions and DNA manipulations: DNA manipulations and yeast transformations were conducted as previously described (KEIL and ROEDER 1984; VOELKEL-MEIMAN, KEIL and ROEDER 1987). We constructed a yeast strain, K2307, that contains three different substrates for assaying intrachromosomal recombination. One of these substrates provides a qualitative colony color sectoring assay for *HOT1* activity. The second substrate is used to quantitate *HOT1* activity and the third substrate measures recombination in the absence of *HOT1*.

To generate the substrate used for the colony color sectoring assay, plasmid pL1161 was constructed by digesting pYeADE5,7(5.2L) (HENIKOFF 1986) with *Eco*RI and religating to produce pL1067. This removes the 2μ, *LEU2* and *Eco*RI-*Bam*HI pBR322 sequences as well as part of the sequences flanking *ADE5,7*. Plasmid pR124 contains a 2.2-kb *Sal*I-*Xho*I fragment of *LEU2* at the *Sal*I site of pBR322. The unique *Bst*EII site in the *LEU2* gene was modified to a *Not*I site by digesting pR124 with *Bst*EII, filling in the 3′ recessed ends with the Klenow fragment of DNA polymerase I and ligating in the presence of *Not*I linkers, to produce *leu2::Not*I in pL1069. The 2.0-kb *Sal*I-*Hpa*I fragment containing *leu2::Not*I, isolated from pL1069, was made blunt ended using the Klenow fragment. This fragment was inserted in the blunt-ended *Eco*RI site of pL1067 to generate pL1100. Plasmid pL1161 was constructed by ligating the 570-bp *Eco*RI fragment containing EI→*HOT1* from pL623 (VOELKEL-MEIMAN, KEIL and ROEDER 1987) into the *Eco*RI site of pL1100 in the orientation shown in Figure 1A.

The plasmid used to construct the substrate to quantitate *HOT1* activity is pL623, which contains a 570 base-pair EI→ *HOT1* subclone, the *URA3* gene and part of the *HIS4* gene in pBR322 (VOELKEL-MEIMAN, KEIL and ROEDER 1987). Plasmids pL623 and pL1161 were digested with *Cla*I and *Not*I, respectively, and cotransformed into K1864 (Table 1). Cells of K1864 produce white, Ura$^-$ colonies. Genomic DNA from red Ura$^+$ transformants was analyzed by Southern blot analysis to identify transformants containing each plasmid properly integrated as a single copy at the appropriate chromosomal locus.

To measure recombination in the absence of *HOT1*, a direct repeat of *LYS2* sequences separated by *CAN1* and pBR322 sequences was constructed. A 3.9-kb *Sal*I-*Xho*I fragment from pJHICAN1 (HOFFMANN 1985) that contains most of the *CAN1* gene was inserted into the unique *Sal*I site of pR430, a plasmid containing the *LYS2* gene (YUAN and KEIL 1990). The resulting plasmid was linearized with *Stu*I targeting it to integrate (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) at the chromosomal *lys2* locus of yeast strain K1844 to produce K1864. Southern blot analysis was used to confirm that the plasmid integrated properly. The strain containing all three of these intrachromosomal recombination substrates, K2307, was used to isoalte *trans*-acting mutants that alter *HOT1* activity.

To measure rDNA recombination, the *URA3* gene was inserted into the tandem array of rDNA repeats. The *Hin*dIII site of pKC7 (RAO and ROGER 1979) was destroyed by digesting with *Hin*dIII, treating with the Klenow fragment and ligating. The 4.6-kb *Bgl*II-A fragment of rDNA (KEIL and ROEDER 1984) was inserted at the unique *Bgl*II site of this plasmid to produce pL915. The 1.2-kb *Hin*dIII fragment containing the *URA3* gene was inserted at the unique *Hin*dIII site of the *Bgl*II-A fragment to make pL938. This plasmid was then digested with *Bgl*II and transformed into *ura3 his4* recombinants of *hrm* and wild-type strains. Southern analysis identified transformants containing a single copy of *URA3* in the rDNA.

To determine the interaction of the *hrm* mutations and *rad52*, substitution transformation (ROTHSTEIN 1983) was used to place the *TRP1*-disruption mutation of *RAD52*, *rad52-8* (obtained from D. Schild), into strains containing the various *hrm* mutations.

Media and growth conditions: Yeast synthetic complete medium (SC), drop-out media (for example SC lacking His, SC-His), medium containing 5-fluoro-orotic acid (SC + 5FOA) and sporulation medium were prepared as described previously (ZEHFUS *et al.* 1990). Medium containing canavanine (SC + Can) was prepared by adding 60 mg of L-canavanine sulfate per liter of SC-Arg medium. YPD was prepared as described previously (SHERMAN, FINK and HICKS 1986). Cryptopleurine (Cry) was added to YPD at 0.5 mg/liter to make YPD + Cry. Medium to enhance the visualization of white sectors in red colonies (SC/color) was prepared as described for SC except that 5% glucose and 5 mg/liter adenine sulfate were used.

In general, strains were grown at 30°. To test for growth

TABLE 1

List of yeast strains

Strain	Genotype[a]	Source
RLK88-3C	*MATa his4-260 ade2-1 ura3-52 leu2-3,112 trp1-HIII lys2-ΔBX can1*	This study
K1844	*MATa his4-260 ade2-1 ade5 ura3-52 leu2-3,112 trp1-HIII lys2-ΔBX can1*	This study[b]
K1864	*MATa his4-260 ade2-1 ade5 ura3-52 leu2-3,112 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1*	This study[b]
DR113-1	*MATα his4-260, ade2-1, ura3-52, leu2-3,112 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1*	This study[b]
K2307	*MATa his4-260::URA3::his4-Δ::HOT1 ade2-1 ade5 ura3-52 HOT1::leu2::ADE5,7::leu2-3,112 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1*	This study[b]
K2302	*MATa his4-260::URA3::his4-Δ ade2-1 ade5 leu2::ADE5,7::leu2-3,112 trp1-HIII lys2-ΔBX::CAN1::LYS2 ura3-52 can1*	This study[b]
g218/4d	*MATα rad50-1 ade1 ade2 lys1 lys2 trp5 tyr1 his can1*	YGSC[c]
M9-2B	*MATα rad51::ΔLEU2 leu2-3,112 his1-7 ura3-52 ade5*	J. GAME
g160/2d	*MATα rad52-1 ade2-1 arg4 arg9 trp1 his5 lys1-1 ilv3 leu2*	YGSC
g151/12a	*MATα rad53-1 ade2-1 ade1 arg4-17 lys1-1 his5-2 t.s.*	YGSC
XS834-20B-*rad54Δ::LEU2*	*MATα rad54Δ::LEU2 leu2-3,112 ura3 his1-1 trp2 can1*	D. SCHILD
STL20-1	*MATα rad55Δ::LEU2 his7 ade5 trp leu2 ura3*	R. MORTIMER
XS834-20B-*rad57Δ::LEU2*	*MATα rad57Δ::LEU2 leu2-3,112 ura 3 his1-1 trp2 can1*	D. SCHILD

[a] The *lys2-ΔBX* and *trp1-HIII* mutations were made as described by YUAN and KEIL (1990).

[b] Derived from RLK88-3C.

[c] Obtained from the Yeast Genetic Stock Center (YGSC) Department of Biophysics and Medical Physics, University of California, Berkeley, California.

at various temperatures, *hrm* mutants were grown in liquid YPD medium overnight and 5 μl of a 1 to 100 dilution was spotted onto each of four YPD plates. One plate was incubated at each of the following temperatures: 23°, 30°, 34° and 37°. To test for gamma-radiation sensitivity, cells were replicated to YPD medium, exposed to 50 krad in a Gammacell 220 Irradiator, and then grown at 30°. To test UV sensitivity, cells were replicated to YPD medium and exposed at 30 erg/mm^2/sec for 1 or 2 min. The irradiated plates were grown in the dark at 30°.

UV mutagenesis: Strain K2307 was used to screen for mutants that affect mitotic recombination. From a saturated overnight culture, about 200 cells per plate were plated on SC/color and irradiated with a germicidal UV light to 50% lethality. These plates were incubated in the dark at 30°. The frequency of white sectors on red colonies was scored after 3 days. Colonies with increased or decreased sectoring were characterized further.

Analysis of spontaneous mutation frequency: After backcrossing twice to wild type, the spontaneous mutation frequency of each *hrm* mutant was measured by growing five independent cultures to saturation in YPD and plating appropriate dilutions on YPD and YPD + Cry. Cryptopleurine-resistant colonies were counted after 5 days at 30°.

Fluctuation tests, statistical analysis, and genetic analysis: At least three cultures from three independent transformants were used to determine recombination frequencies. Fluctuation tests to assay *HOT1* activity were conducted as described previously (YUAN and KEIL 1990). The frequency of mitotic recombination in rDNA is high. To assay the frequency of rDNA exchange, single colonies from rDNA::*URA3* strains were isolated from SC-Ura plates and grown to saturation in liquid SC-Ura medium. The frequency of Ura$^-$ recombinants was determined by plating appropriate dilutions of cells on SC + 5FOA and SC media. Statistical analysis was performed as described previously (YUAN and KEIL 1990). Tetrad analysis was conducted by standard procedures (SHERMAN, FINK and HICKS 1986).

RESULTS

Isolation of mutants affecting the recombination-stimulatory activity of *HOT1*: To isolate mutants that affect *HOT1* activity, we constructed a strain, K2307, that contains three substrates for intrachromosomal recombination.

Qualitative assay for HOT1 activity (colony color sectoring assay): To assay recombination in large numbers of mutagenized cells, we used a colony color sectoring assay to directly visualize the frequency of white sectors in a red colony. The recombination substrate for the sectoring assay, *HOT1::leu2::ADE5,7::leu2*, contains directly repeated *leu2* genes separated by *ADE5,7* and pBR322 sequences on chromosome *III* (Figure 1A). In yeast, *ade2* cells are red, while *ade2 ade5* cells are white (ROMAN 1956). Colonies of strain K2307 (*ade2 ade5 ADE5,7*) are red with white sectors (Figure 2A). The cells in the white sectors (*ade2 ade5*) result from a *HOT1*-stimulated recombination event between the *leu2* repeats that excises the *ADE5,7* gene, pBR322 sequences and one of the *leu2* genes (Figure 1A). The frequency of white sectors gives a qualitative indication of *HOT1* activity.

Quantitative HOT1 assay: The strain that contains the colony color sectoring assay also contains a second recombination substrate, the *HOT1::his4::URA3::his4* duplication (Figure 1B). The frequency of either Ura$^-$ or His$^+$ recombinants can be measured to assay the effect of *hrm* mutations on *HOT1* activity (Figure 1B). Identifying potential mutants using the colony color sectoring assay and then examining recombination of the *HOT1::his4::URA3::his4* duplication permits us to

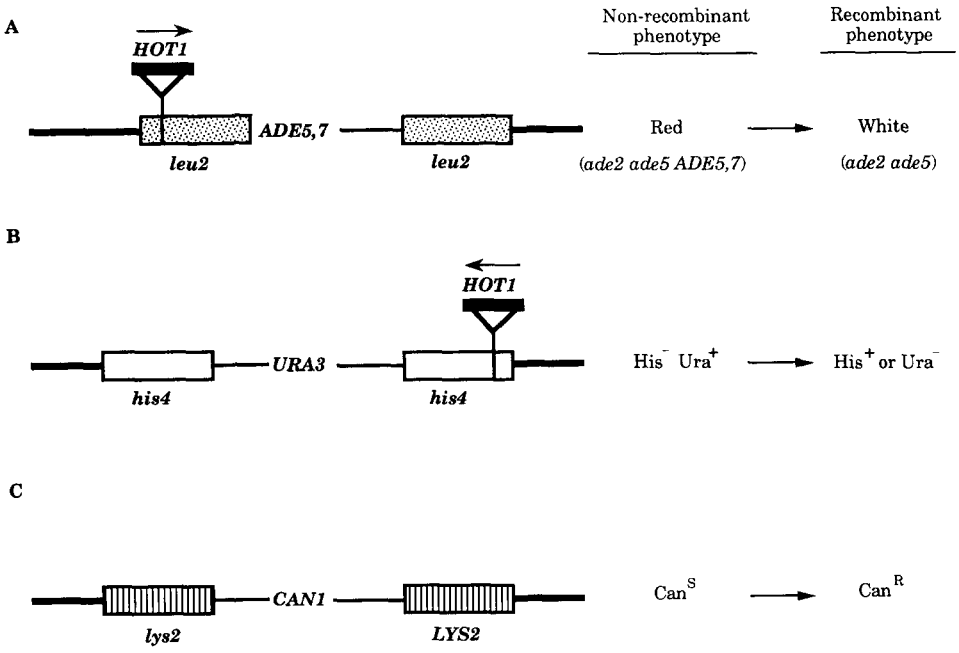

FIGURE 1.—The recombination substrates used to isolate *trans*-acting *HOT1* recombination mutations. Three duplications were constructed in the strain K1844 (*MAT***a** *his4-260 ade2-1 ade5 ura3-52 leu2-3,112 trp1-HIII lys2-ΔBX can1*). One duplication (A) contains *ADE5,7* and pBR322 sequences flanked by two *leu2* mutations on chromosome *III*. A 570-bp EI→*HOT1* fragment was inserted in one of the copies of *leu2*. Nonrecombinant cells, which contain this duplication, are red. Excisive recombinants, which lose the *ADE5,7* gene, are white. The second duplication (B) contains two different *his4* mutations separated by pBR322 and *URA3* sequences on chromosome *III* as described previously (VOELKEL-MEIMAN, KEIL and ROEDER 1987). One of the *his4* genes contains the EI→*HOT1* fragment. The frequency of both His$^+$ and Ura$^-$ recombinants can be measured for this duplication. The third duplication (C) contains *CAN1* and pBR322 sequences flanked by *lys2* sequences on chromosome *II*. *HOT1* is absent from this duplication. Recombinants that lose *CAN1*, pBR322 and the *lys2* (or *LYS2*) repeat are Can^R. Heavy line: chromosomal DNA; black box: 570 bp EI→subclone of *HOT1*; dotted box: *leu2* gene; open box: *his4* gene; striped box: *lys2* or *LYS2* gene; thin line: pBR322 sequences; the arrow: direction of transcription initiated at the 35S ribosomal RNA transcription initiation site.

rapidly eliminate a number of uninteresting events that can occur. Examples of such events include: ectopic recombination of the *ADE5,7* gene used in the colony color sectoring assay with the *ade5* gene at the normal chromosomal location; duplication of the *ADE5,7* gene by unequal-sister-chromatid exchange of the *leu2* repeats; and gene conversion between the *leu2* repeats resulting in loss of *HOT1* from this duplication. Using two different assays for *HOT1* activity also eliminates the recovery of *cis*-acting mutations in *HOT1*.

Assay for non-HOT1-stimulated recombination: To obtain a preliminary indication of whether the mutants specifically affect *HOT1* activity, a duplication of *lys2* genes separated by *CAN1* and pBR322 sequences, *lys2::CAN1::LYS2*, was constructed on chromosome *II* (Figure 1C). *HOT1* is not present in this duplication. Non-*HOT1* recombination is measured by the frequency of canavanine-resistant (Can^R) recombinants.

Isolation and preliminary characterization of hypo-recombination mutants that affect *HOT1* activity: Strain K2307, which contains all three of the duplications described above, was mutagenized by UV and the colonies derived from surviving cells were screened by the colony color sectoring assay. Mutants with decreased or increased numbers of sectors were identified (Figure 2, B and C, respectively). Both types of mutants were patched on YPD and replicated to SC-His, SC + 5FOA and SC + Can to examine the degree of papillation, which reflects the frequency of recombination. Mutants that increased or decreased the frequency of His$^+$ or Ura$^-$ recombinants were further examined by Southern blot analysis to confirm that they still contained the proper configuration of the duplications after mutagenesis. From approximately 20,000 colonies seven hypo-recombination mutants and twelve hyper-recombination mutants were isolated. Further characterization of the seven hypo-recombination mutants is described below.

The effect of the hypo-recombination mutants on the recombination-stimulatory activity of *HOT1* is shown in Table 2. These mutants decrease the frequency of both His$^+$ and Ura$^-$ recombinants but do not significantly affect the frequency of Can^R recombinants giving a preliminary suggestion that these mutants specifically affect *HOT1*-stimulated exchange.

As shown in Table 2, growth of two of these mutants, *hrm3* and *hrm4*, is affected by temperature. *Hrm3* does not grow at 37°, but, unlike *cdc* mutants, the cells do not arrest at a specific stage of the cell-

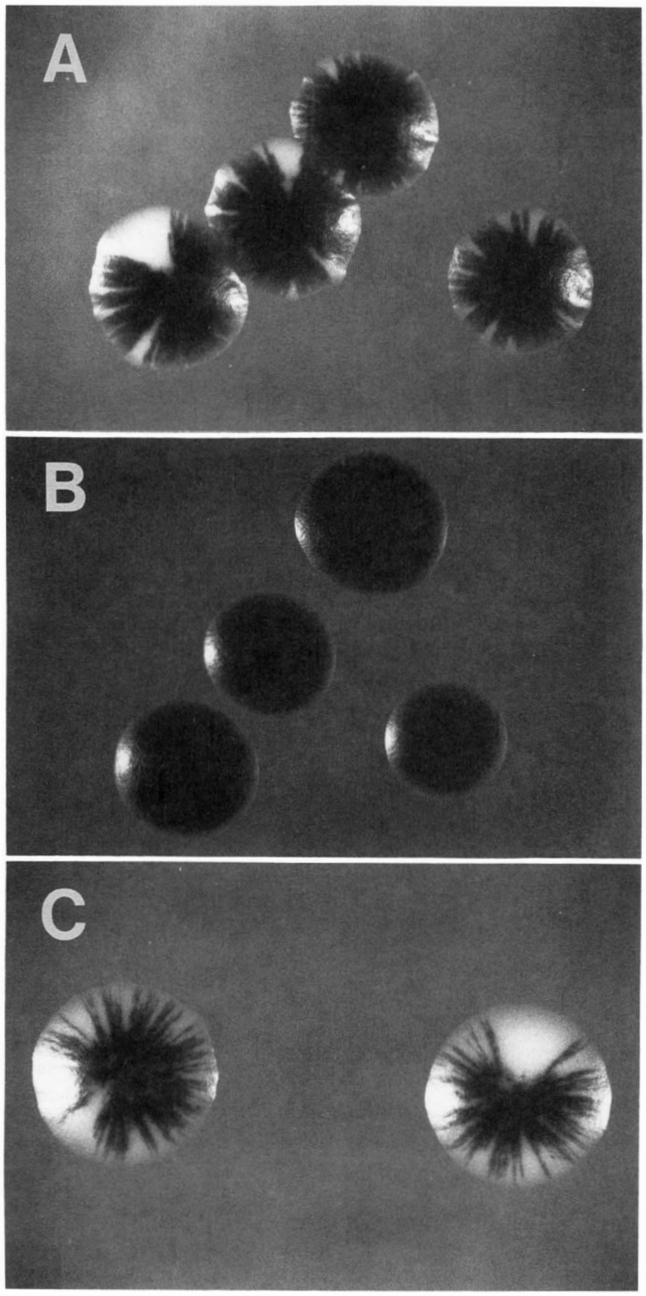

FIGURE 2.—Sectoring of wild-type and mutant colonies. The strain K2307 (*MAT***a** *HOT1::leu2::ADE5,7::leu2-3 112 HOT1::his4-Δ::URA3::his4-260 ura3-52 lys2-ΔBX::CAN1::LYS2 ade2-1 ade5 trp1-HIII can1*) contains a duplication (*HOT1::leu2::ADE5,7::leu2*) for the colony color sectoring assay. Each colony of the wild-type strain (A) produces approximately 10–20 white sectors. The formation of white sectors results from excisive recombination between the two *leu2* genes and is dependent on *HOT1* activity. Colonies of strains containing a hypo-recombination mutation (B) have very few white sectors. Colonies of hyper-recombination mutants (C) produce numerous white sectors.

division cycle when incubated at 37°. *Hrm4* grows poorly at both 23° and 37°. *Hrm2*, *hrm3* and *hrm4* grow slowly at 30°, forming colonies about one-half the diameter of wild-type colonies after 2 days. *Hrm5-1* and *hrm5-2* are sensitive to γ irradiation. Complementation analysis with previously characterized

TABLE 2

Characterization of *HOT1* hypo-recombination mutants

	Preliminary frequency[a]							
	HOT1		Non-*HOT1*					
	His^+	Ura^-	Can^R	Growth at			Sensitivity to	
Genotype	(×10⁴)	(×10⁴)	(×10⁴)	23°	30°	37°	UV	γ-Rays
Wild type	28.6	94.9	12.6	++	++	++	++	++
hrm1-1	3.7	4.2	8.3	++	++	++	++	++
hrm1-2	9.1	9.0	8.0	++	++	++	++	++
hrm2	2.0	4.6	5.9	+	+	+	++	++
hrm3	4.2	38.2	7.5	+	+	−	++	++
hrm4	5.6	25.6	9.6	±	+	±	++	++
hrm5-1	0.5	2.0	12.3	++	++	++	++	−
hrm5-2	6.2	20.1	31.4	++	++	++	++	−

[a] The preliminary frequencies of His^+, Ura^- and Can^R were not tested for statistic significance since only three cultures were assayed for each strain. Data for statistical analysis is shown in Table 3.

++ = wild-type response, + = slow growth, ± = poor growth, − = no growth.

γ-ray-sensitive mutations showed that both *hrm5-1* and *hrm5-2* fail to complement *rad52* and, therefore, are *RAD52* alleles. ZEHFUS *et al.* (1990) have shown that *rad52* decreases the activity of *HOT1*. Isolation of mutations in *RAD52* confirms the utility of this screen in identifying genes involved in recombination. None of these *hrm* mutants increases sensitivity to UV irradiation.

To further characterize the effect of these mutations on DNA repair, the frequency of spontaneous mutants in the *hrm* mutant strains was examined. *Hrm4* has a significant fourfold decrease in the frequency of spontaneous resistance to cryptopleurine (data not shown). The other four mutants (*hrm1-1*, *hrm1-2*, *hrm2* and *hrm3*) do not significantly affect the frequency of spontaneous mutants.

Genetic analysis of hypo-recombination mutants: Genetic analysis was conducted on the five hypo-recombination mutations that are not sensitive to gamma irradiation (*hrm1-1*, *hrm1-2*, *hrm2*, *hrm3* and *hrm4*). When backcrossed to wild type, each of these mutations segregated 2:2 for the recombination phenotype indicating that the mutations are in single nuclear genes. The temperature sensitivity or growth defects of *hrm2*, *hrm3* and *hrm4* cosegregated with the decreased recombination phenotype. Analysis of the phenotypes of heterozygous diploids shows that all of the phenotypes of the *hrm* mutations are recessive.

Tetrad analysis was performed on pairwise crosses between the mutants. When *hrm1-1* was crossed to *hrm1-2*, only parental ditype tetrads were observed among 22 tetrads examined. The absence of recombination indicates that the *hrm1-1* and *hrm1-2* mutations are either in the same gene or very closely linked genes (<2 map units). The two mutations fail to complement and, therefore, appear to be mutations in the

TABLE 3

Interaction of *rad52* and *hrm* mutations

Genotype	Frequency (×10⁴)		
	HOT1		Non-*HOT1*
	His⁺	Ura⁻	Can^R
Wild type	35.8	137.0	7.9
rad52	0.3	3.0	6.0
hrm1-1	1.9	6.2	8.8
hrm1-1 rad 52	0.3	2.4	10.0
hrm1-2	3.6	16.8	7.5
hrm1-2 rad52	0.2	1.0	8.4
hrm2	2.8	11.6	4.9
hrm2 rad52	0.2	2.2	5.9
hrm3	7.0	27.1	7.2
hrm3 rad52	0.4	1.6	6.9
hrm4	4.6	17.3	4.9
hrm4 rad52	0.4	1.4	2.8

There is no statistically significant difference between the recombination frequency for the *rad52* strains and any of the *hrm rad52* strains.

TABLE 4

Effect of *HOT1* hypo-recombination mutations on mitotic intrachromosomal recombination

Genotype	Frequency (×10⁴)				
	HOT1		Non-*HOT1*		rDNA
	His⁺	Ura⁻	His⁺	Ura⁻	Ura⁻
Wild type	34.1	312.0	1.9	1.9	9.6
hrm1-1	1.7**	1.7**	1.5	0.6	0.5**
hrm1-2	2.4**	1.6**	2.0	1.2	0.2**
hrm2	2.6**	5.7**	1.0	0.9	2.5**
hrm3	4.5**	20.4**	1.2	1.4	0.2**
hrm4	6.2**	22.5**	3.1	2.0	12.6

** $P < 0.01$ as compared to wild type.

same gene. This gene (*hrm1*) is located approximately 40 cM from *RAD52* as estimated from analysis of crosses with *rad52* strains. Genetic analysis showed that *hrm1* is unlinked to *hrm2*, *hrm3* and *hrm4*.

Crosses of *hrm4* strains with either *hrm2* or *hrm3* strains show that *hrm4* is unlinked to either *hrm2* or *hrm3*. Crosses of *hrm2* and *hrm3* strains gave low spore viability (58%). Only one of 35 tetrads had four viable spores. Double mutant spores, *hrm2 hrm3*, may be inviable. From the viable spores, a number of colonies showed wild-type levels of *HOT1* activity indicating that *hrm2* and *hrm3* identify different genes. These two mutations complement each other in heterozygous diploids further demonstrating that they define different genes.

Epistatic interaction of *hrm* mutations with *rad52*: The interaction of two mutations affecting recombination can be studied by constructing double-mutant strains (Cox and Game 1974). The type of interaction, epistatic, additive or synergistic, helps define whether two mutations affect the same or different recombination pathways. The interaction of each of the *hrm* mutations with *rad52* was analyzed both for *HOT1*-stimulated recombination (*HOT1::his4::URA3::his4*) and non-*HOT1*-stimulated recombination (*lys2::CAN1::LYS2*). As shown in Table 3, *rad52* decreases the frequency of His⁺ and Ura⁻ recombinants more than any of the *hrm* mutations. For example, in *rad52* strains the frequency of Ura⁻ recombinants is decreased 46-fold, while in the *hrm* strains this frequency is decreased 5–22-fold. Strains containing both *rad52* and any of the *hrm* mutations have the same frequency of His⁺ and Ura⁻ recombinants as the *rad52* strains. Thus, there is an epistatic interaction between *rad52* and each of the *hrm* mutations with respect to the recombination-stimulatory activity of *HOT1*. The frequency of Can^R recombinants is not affected in any of the single or double mutants.

***Hrm1* through *hrm4* specifically affect *HOT1* activity:** Preliminary characterization of these *hrm* mutants indicates that all of them, including *hrm5* (allelic to *rad52*), specifically affect *HOT1* activity based on exchange in the *lys2::CAN1::LYS2* duplication (Table 2). As shown above, recombination to produce Can^R cells in this duplication is not affected by the *TRP1* disruption allele of *rad52*. L.-W. Yuan and R. L. Keil (unpublished results) find that mitotic intrachromosomal recombination of various duplications is affected differently by *rad52*. Zehfus *et al.* (1990) have previously shown that recombination in the *his4::URA3::his4* duplication lacking *HOT1* is significantly decreased by *rad52*. To further assess whether the *hrm* mutations specifically alter *HOT1* activity, *ura3 his4* recombinants from the *hrm* and wild-type strains were transformed to produce the *his4::URA3::his4* duplication lacking *HOT1*. *Hrm1* through *hrm4* do not significantly affect the frequency of exchange in this duplication (Table 4), indicating that they specifically affect recombination in this duplication when *HOT1* is present. To confirm that the *hrm* mutations are *trans*-acting mutations, these *ura3 his4* strains were also transformed to reconstruct the *HOT1::his4::URA3::his4* duplication. All of the *hrm* strains still show significantly decreased *HOT1* activity (Table 4). This demonstrates that the *hrm* strains contain *trans*-acting mutations and not lesions in the *HOT1* sequence.

***Hrm1*, *hrm2* and *hrm3* affect rDNA recombination:** Since *HOT1* is derived from the rDNA repeat unit, Keil and Roeder (1984) proposed that *HOT1* may be important for maintaining homogeneity among the tandem rDNA repeats by promoting recombination in the array. We investigated the effect of the *hrm* mutations on rDNA recombination. Mitotic intrachromosomal rDNA exchange is measured by the frequency of loss of a *URA3* gene inserted in the array of repeats. *Hrm1-1*, *hrm1-2* and *hrm3* dramatically decrease the frequency of Ura⁻ recombinants

(Table 4). The frequency of rDNA recombination in *hrm2* is slightly but significantly reduced (approximately fourfold). *Hrm4* does not significantly affect the frequency of rDNA exchange. Growth of *hrm4* strains at 34°, a temperature permitting very slow growth of these mutants, did not significantly affect the frequency of rDNA recombination as compared to wild type (data not shown).

DISCUSSION

We have developed a method to isolate *trans*-acting mutations that alter the recombination-stimulatory activity of *HOT1*, a recombination hotspot derived from the rDNA repeat of yeast. Both hypo-recombination and hyper-recombination mutants have been isolated. Seven mutations that decrease the recombination-stimulatory activity of *HOT1* were characterized. These seven mutations identify recessive nuclear genes. Two new alleles of *RAD52* (*hrm5-1* and *hrm5-2*) were isolated demonstrating that genes involved in recombination can be identified by this screen. The other four genes identified appear to be novel. All four of them specifically decrease *HOT1* activity and three of them decrease exchange in rDNA.

Studying the effects on recombination of the *hrm* mutations provides new information about the genetic control of *HOT1*-stimulated recombination, rDNA exchange and recombination that occurs in the absence of *HOT1*. The *hrm1*, *hrm2* and *hrm3* mutations are unique in that they affect *HOT1*-stimulated exchange as well as rDNA recombination. They do not affect recombination in the absence of *HOT1*. This finding provides the first evidence that *HOT1* may play a role in rDNA recombination.

Previously, several mutations that enhance rDNA recombination were identified. These include *top1* and *top2*, DNA topoisomerase mutations (CHRISTMAN, DIETRICH and FINK 1988), *sir2*, a mutation affecting the expression of the mating-type loci (GOTTLIEB and ESPOSITO 1989), *rrm1* (C. L. PINKOWSKI and R. L. KEIL, unpublished results) and *rrm3*, a mutation that also affects exchange in the tandem repeats at *CUP1* (R. L. KEIL and A. D. MCWILLIAMS, unpublished results). None of these mutations affect *HOT1* activity (K. VOELKEL-MEIMAN and G. S. ROEDER, unpublished results; S. GOTTLIEB, G. S. ROEDER and R. L. KEIL, unpublished results; R. L. KEIL, A. D. MCWILLIAMS and C. L. PINKOWSKI, unpublished results). CHRISTMAN, DIETRICH and FINK (1988) suggested that such genes may suppress rDNA exchange in wild-type strains. Our finding that some mutations decrease rDNA recombination suggests that the normal level of rDNA exchange may be the result of an interaction between the recombination-stimulatory activity of *HOT1* and suppression of exchange by a number of gene products.

The *hrm4* mutation decreases *HOT1*-stimulated exchange but does not affect recombination in the rDNA or in the absence of *HOT1*. It is possible that the gene product is specifically required for *HOT1*-stimulated recombination occurring outside of rDNA where additional factors may be required.

The recombination-stimulatory activity of *HOT1* appears to be related to its ability to promote high levels of RNA polymerase I transcription. Therefore, products of the *HRM* genes may be directly involved in *HOT1* recombination or they may be required for 35S rRNA transcription. The temperature-sensitive phenotype of *hrm3* and its affect on rDNA exchange make this a candidate for a gene involved in 35S rRNA transcription. Interestingly, *hrm2* and *hrm3* decrease rDNA recombination significantly when cells are grown in SC-Ura medium but not when cells are grown in SC medium. This difference does not result from overgrowth or selective death of Ura^- or Ura^+ cells in either medium (our unpublished data). The reason for this difference is unclear. It may result from a difference in RNA metabolism in various media. Further genetic and molecular analysis of these *trans*-acting genes and their products will lead to an understanding of the mechanism by which *HOT1* stimulates recombination and its role in rDNA exchange.

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