Gene Conversion Tracts Stimulated by *HOT1*-Promoted Transcription Are Long and Continuous

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ABSTRACT

The recombination-stimulating sequence, *HOT1*, corresponds to the promoter of transcription by yeast RNA polymerase I. The effect of *HOT1* on mitotic interchromosomal recombination was examined in diploid strains carrying a heterozygous *URA3* gene on chromosome *III*. The frequency of Ura$^-$ recombinants was increased 20-fold when *HOT1* was inserted into the chromosome *III* copy marked with *URA3*, at a location 48 kbp centromere-proximal to *URA3*. Ura$^-$ recombinants were increased only 2-fold when *HOT1* and *URA3* were on opposite homologues. These results suggest that most *HOT1*-promoted Ura$^-$ recombinants result from gene conversion and that sequences on the *HOT1*-containing chromosome are preferentially converted. Characterization of Ura$^-$ recombinants isolated from strains carrying multiple markers on chromosome *III* indicates that *HOT1*-promoted gene conversion tracts are unusually long (often >75 kbp) and almost always continuous. Furthermore, conversion tracts frequently extend to both sides of *HOT1*. We suggest that *HOT1* promotes the formation of a double-strand break which is often followed by exonucleolytic digestion. Repair of the broken chromosome could then result from gap repair or from replicative repair primed only by the centromere-containing chromosomal fragment.

THE recombination-stimulating sequence, *HOT1*, is derived from the ribosomal DNA of *Saccharomyces cerevisiae* and corresponds to sequences required for promotion of transcription by RNA polymerase I (VOELKEL-MEIMAN, KEIL and ROEDER 1987; STEWART and ROEDER 1989). When inserted at novel locations in the yeast genome, *HOT1* stimulates both intrachromosomal and interchromosomal recombination in mitotically dividing cells (KEIL and ROEDER 1984; VOELKEL-MEIMAN, KEIL and ROEDER 1987; VOELKEL-MEIMAN and ROEDER 1990). Several observations indicate that transcription by RNA polymerase I initiating in *HOT1* and proceeding through the adjacent sequences is responsible for the enhancement of recombination (VOELKEL-MEIMAN, KEIL and ROEDER 1987; STEWART and ROEDER 1989).

We have previously examined the effect of *HOT1* on mitotic interchromosomal recombination (VOELKEL-MEIMAN and ROEDER 1990). *HOT1* was inserted just upstream of the *HIS4* gene on chromosome *III* in diploid strains carrying *his4* heteroalleles. *HOT1* stimulated the formation of *His*$^+$ prototrophs resulting from gene conversion when present on one or both copies of the chromosome. When *HOT1* was present on only one of the two homologs, the *his4* gene on the *HOT1*-containing chromosome was preferentially converted. Thus, *HOT1* defines the chromosome on which it resides as the recipient of genetic information during conversion. In this respect, *HOT1* is similar to sequences that stimulate meiotic recombination in *S. cerevisiae* (NICOLAS *et al.* 1989), *Schizosaccharomyces pombe* (GUTZ 1971), *Sordaria brevicolis* (MACDONALD and WHITEHOUSE 1979) and *Neurospora crassa* (ANGEL, AUSTIN and CATCHESIDE 1970).

In this paper, we have extended our studies of *HOT1*-promoted interchromosomal recombination. Our results indicate that *HOT1* stimulates the formation of recombinants that are homozygous for markers distal to the site of *HOT1* insertion. In *HOT1* heterozygotes, sequences present on the *HOT1*-containing chromosome are preferentially lost and sequences on the homolog are duplicated. Furthermore, these long, continuous conversion tracts frequently include a marker that lies centromere-proximal to *HOT1* even when *HOT1*-promoted transcription proceeds away from the centromere. These results suggest that events initiating in the region of *HOT1*-promoted transcription can extend a significant distance (25–50 kbp) to both sides of the initiation site. We propose that *HOT1*-promoted transcription stimulates the formation of a double-strand break which is subsequently repaired by a "break-and-replicate" pathway of recombination. In this pathway, sequences distal to the break are lost and the broken chromosome is repaired by replication using the centromere-containing chromosomal fragment as primer and the homologous chromosome as template.

MATERIALS AND METHODS

Media: SC, MIN, GNA and YEPD media were prepared as described by SHERMAN, FINK and HICKS (1986). SC + 5-

TABLE 1

Yeast strains

Strain	Genotype
RLK1-3C	*MATα his4-260 ade2-1 ura3-52*
K144	*MATa leu2-112 his4-712 ura3-52*
SR202-3B	*MATa leu2-112 his4-260 ade2-1*
JE138-17B	*MATα leu2 his4-260 ura3 arg4 spo13::URA3 thr1-4 trp1 lys2*
M520	*MATa leu2 his4-712 ade2 ura3 spo13::URA3 trp1 thr1-4 lys2*
M521	*MATα leu2 his4-712 ade2 spo13::URA3 thr1-4 lys2*
S1243	*MATa leu2-112 his4-712 HMLα ADE2 ura3-52* *MATα LEU2 his4-620 HMLα[URA3] ade2-1 ura3-52*
S1245	*MATa leu2-112 his4-712 HMLα[URA3] ADE2 ura3-52* *MATα LEU2 his4-260 HMLα ade2-1 ura3-52*
S1250	*MATa leu2-112 bik1::HOT1-t his4-712 HMLα[URA3] ADE2 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 HMLα ADE2::BIK1::ade2 ura3-52*
S1254	*MATa leu2-112 bik1::HOT1-t his4-712 HMLα ADE2 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 HMLα[URA3] ADE2::BIK1::ade2 ura3-52*
S1274	*MATa leu2-112 bik1::HOT1-t his4-712 HMLα ADE2 ura3-52* *MATα LEU2 BIK1 his4-260 HMLα[URA3] ADE2::BIK1::ade2 ura3-52*
S1278	*MATa leu2-112 bik1::HOT1-t his4-712 HMLα[URA3] ADE2 ura3-52* *MATα LEU2 BIK1 his4-260 HMLα ADE2::BIK1::ade2 ura3-52*
S1282	*MATa leu2-112 bik1::hot1-t his4-712 ADE2 TRP1 ura3-52* *MATα LEU2 BIK1 his4-260 J10A::URA3 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1289	*MATa leu2-112 bik1::hot1-t his4-712 J10A::URA3 ADE2 TRP1 ura3-52* *MATα LEU2 BIK1 his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1291	*MATa leu2-112 bik1::HOT1-c his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-c his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1293	*MATa leu2-112 his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-c his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1294	*MATa leu2-112 bik1::HOT1-c his4-712 B9G::TRP1 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 BIK1 his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1295	*MATa leu2-112 bik1::HOT1-c his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1391	*MATa leu2-112 his4-712 ADE2 trp1-H3 ura3-52* *MATα LEU2 his4-260 J10A::URA3 ade2-1 trp1-H3 ura3-52*
S1392	*MATa leu2-112 his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 his4-260 ade2-1 trp1-H3 ura3-52*
S1393	*MATa leu2-112 bik1::HOT1-t his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1394	*MATa leu2-112 bik1::HOT1-t his4-712 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 J10A::URA3 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1395	*MATa leu2-112 BIK1 his4-712 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 J10A::URA3 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1396	*MATa leu2-112 BIK1 his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1397	*MATa leu2-112 bik1::HOT1-t his4-712 ADE2 trp1-H3 ura3-52* *MATα LEU2 BIK1 his4-260 J10A::URA3 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1398	*MATa leu2-112 bik1::HOT1-t his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 BIK1 his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1399	*MATa leu2-112 his4-712 B9G::TRP1 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 his4-260 ade2-1 trp1-H3 ura3-52*
S1402	*MATa leu2-112 bik1::HOT1-t his4-712 B9G::TRP1 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 BIK1 his4-260 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1411	*MATa leu2-112 his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 his4-260 B9G::TRP1 ade2-1 trp1-H3 ura3-52*
S1413	*MATa leu2-112 BIK1 his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 bik1::HOT1-t his4-260 B9G::TRP1 ADE2::BIK1::ade2 trp1-H3 ura3-52*
S1414	*MATa leu2-112 bik1::HOT1-t his4-712 J10A::URA3 ADE2 trp1-H3 ura3-52* *MATα LEU2 BIK1 his4-260 B9G::TRP1 ADE2::BIK1::ade2 trp1-H3 ura3-52*

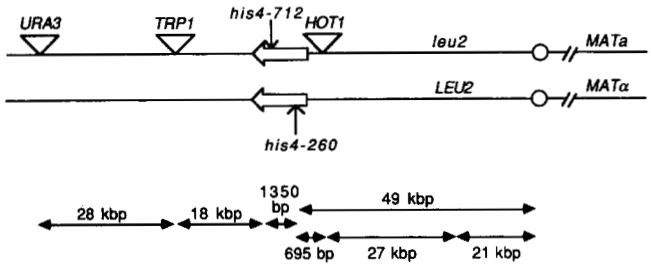

FIGURE 1.—Distribution of markers on chromosome *III*. Shown at the top is the distribution of chromosome *III* markers in parental diploids used to measure recombination. The triangles indicate the sites of insertion of *URA3*, *TRP1* and *HOT1*. In S1402, these markers are present on the *his4-712* chromosome as diagrammed. The inserts present in other strains and the chromosome *III* homolog on which they reside are indicated in Table 1. The open arrow indicates the position and orientation of the *HIS4* gene. The circle indicates the chromosome *III* centromere. Shown below the chromosomes are the physical distances between markers. The *his4-260* and *his4-712* mutations lie 45 bp and 1396 bp, respectively, downstream of the start of the *HIS4* coding region (DONAHUE, FARABAUGH and FINK 1982). *HOT1* is inserted 650 bp upstream of *HIS4*. The sizes given for the *URA3-TRP1*, *TRP1-his4-712*, *HOT1-LEU2*, and *LEU2-CEN3* intervals are approximate and are based on the data of NEWLON *et al.* (1986). The distance between *URA3* and the left end of the chromosome is 19 kbp (BUTTON and ASTELL 1986). Diagram is not to scale.

fluoro-orotic acid (FOA) was made by supplementing SC to 0.5 mM uracil and 0.88% FOA (BOEKE, LACROUTE and FINK 1984).

Yeast strains and transformations: All of the strains used to measure recombination are isogenic diploids derived from two haploid parents, RLK1-3C and K144 (Table 1). Derivatives of these strains were constructed by transformation using the method of ITO *et al.* (1983). Transformants were verified by Southern blot analysis (MANIATIS, FRITSCH and SAMBROOK 1982).

HOT1 was inserted as a 570-bp *Bgl*II fragment consisting of the 320-bp *Eco*RI-*Hpa*I fragment containing the enhancer of transcription by RNA polymerase I and a 250-bp *Sma*I-*Eco*RI fragment containing the 35S rRNA initiation site (VOELKEL-MEIMAN, KEIL and ROEDER 1987). A *HOT1* mutant (#38) defective in the stimulation of recombination was constructed by STEWART and ROEDER (1989). The plasmids and procedures used to construct strains carrying wild-type and mutant *HOT1* sequences oriented such that transcription by RNA polymerase I should proceed toward the telomere (*HOT1-t* and *hot1-t*) were described previously (VOELKEL-MEIMAN and ROEDER 1990).

Strains carrying *HOT1* oriented toward the centromere were constructed using plasmids L685 and V106. pL685, described by VOELKEL-MEIMAN, KEIL and ROEDER (1987), was used to transform K144 and its derivatives; pV106 was used to transform RLK1-3c and its derivatives. pV106 is the same as pV127 (VOELKEL-MEIMAN and ROEDER 1990) except that the *Bgl*II fragment containing *HOT1* is derived from pL685 and is inserted in the same orientation as that in pL685. Both plasmids were targeted for integration at *HIS4* by cleavage with *Cla*I and Ura$^+$ transformants were selected. Ura$^-$ segregants which had lost the plasmid due to intrachromosomal recombination were then selected on SC + FOA. These segregants were examined by Southern blot analysis to identify those that retained *HOT1*.

The insertion of *HOT1* sequences results in disruption of the *BIK1* gene (TRUEHEART, BOEKE and FINK 1987). This disruption was complemented by integration of a plasmid containing *BIK1* and *ADE2* at the *ADE2* locus on chromosome *XV* (VOELKEL-MEIMAN and ROEDER 1990).

The *URA3* gene was inserted at two different locations centromere-distal to *HIS4* as described by VOELKEL-MEIMAN and ROEDER (1990). In strains designated as *HMLα[URA3]*, *URA3* is inserted at a *Hin*dIII site immediately centromere-proximal of the *HMLα* locus. In strains designated *J10A::URA3*, *URA3* is inserted into the *Bam*HI fragment that lies immediately adjacent to the *Bam*HI fragment containing *HMLα* (NEWLON *et al.* 1986). The distance between the two sites of *URA3* insertion is about 4.5 kbp. The *URA3* gene was introduced by the one-step gene disruption method of ROTHSTEIN (1983).

The *TRP1* gene was inserted into chromosome *III* using plasmid V163. pV163 is a derivative of plasmid B9G which contains a 6.6 kbp *Bam*HI fragment of chromosome *III* DNA (NEWLON *et al.* 1986). *TRP1* was inserted at a site (either *Rsa*I or *Hin*cII) located about 1.5 kbp away from the centromere-proximal *Bam*HI site. The *TRP1* gene was inserted as an 820-bp *Eco*RI-*Stu*I fragment after filling in the *Eco*RI end with Klenow fragment. pV163 was digested with *Bam*HI prior to transformation into yeast resulting in substitutive transformation (ROTHSTEIN 1983).

For each of the constructions described, three independent transformants were isolated from each haploid starting strain. For strains derived by multiple transformation steps, three independent transformants were isolated after the first transformation. Each of these was then transformed and a single transformant of each was chosen at each of the subsequent steps. The final three *MAT***a** transformants were mated to the three *MATα* transformants in all possible pairwise combinations to generate nine independent diploids.

Fluctuation tests: Fluctuation tests were carried out as described by VOELKEL-MEIMAN and ROEDER (1990) except that cells were plated on SC + FOA instead of SC − His. The method of the median was used to calculate recombination frequencies (LEA and COULSON 1949).

Isolation and characterization of independent His$^+$ and Ura$^-$ recombinants: A protocol similar to the fluctuation test was used to recover independent Ura$^-$ and His$^+$ recombinants and to estimate recombination frequencies. The nine independent diploids with the same genotype were grown to saturation in 2 ml of GNA. Each of these cultures was then diluted and used to set up several 0.5 ml GNA cultures at 30 cells/ml. To isolate independent recombinants, the cells from saturated cultures were spun down and resuspended in 1 ml of water and then ten μl of each was spotted on SC + FOA or SC − His. A single Ura$^-$ or His$^+$ colony was picked and purified from each spot. To estimate the frequency of Ura$^-$ recombinants for the experiment shown in Table 4B, saturated cultures were diluted a thousandfold and 20 μl of each was then patched on an SC + FOA plate.

Ura$^-$ derivatives of S1399, S1402 and S1294 were tested for their genotype at *HIS4* as follows. Recombinants were

Footnote to Table 1

J10A::URA3 indicates an insertion of the *URA3* gene 46 kbp centromere-distal to *HIS4*; *HMLα[URA3]* indicates an insertion of *URA3* immediately adjacent to the *HML* locus (about 4.5 kbp further from *HIS4* than the site of the *J10A::URA3* insertion). *B9G::TRP1* indicates an insertion of the *TRP1* gene 18 kbp distal to *HIS4*. *ADE2::BIK1::ade2* indicates a duplication of the *ADE2* gene flanking pBR322 and *BIK1*. The *trp1-H3* mutation results from a fill-in of the *Hin*dIII site in the *TRP1* coding region.

patched on YEPD medium and then replica plated to SC − His medium and UV-irradiated to induce mitotic recombination (SHERMAN, FINK and HICKS 1986). His⁻ recombinants that gave rise to His⁺ prototrophs during mitotic growth were assumed to be *his4-712/his4-260* in genotype. Ura⁻ recombinants that were phenotypically His⁺ were sporulated and tetrads were dissected (SHERMAN, FINK and HICKS 1986); the *his4* alleles present in His⁻ spore clones were determined by mitotic recombination tests as described previously (VOELKEL-MEIMAN and ROEDER 1990). His⁻ recombinants that failed to generate His⁺ recombinants were force mated to *his4-260* and *his4-712* testers and the resulting triploids were tested for their ability to produce His⁺ recombinants as follows. First, patches of His⁻ recombinants were replica plated to 4 YEPD plates and UV-irradiated to induce homozygosity at the mating type locus. After a 4–6-hr incubation, each YEPD plate was sprayed with a suspension of cells from one of the four tester strains (SR202-3B, JE138-17B, M520 and M521; Table 1). After incubation overnight to allow mating, the plates were replicated to MIN + Leu + Trp + His medium to select for the triploids resulting from mating. After growth at 30° for 2–3 days, the patches of triploids were replicated to medium lacking histidine and UV-irradiated. His⁻ recombinants homozygous for the *his4-260* allele produced His⁺ recombinants only when mated to a *his4-712* tester and vice versa. Recombinants that produced His⁺ recombinants when mated to the *his4-712* tester but not the *his4-260* tester could be either *his4-260/his4-260* or *his4-260/his4-260, 712* in genotype. Several of these recombinants were further characterized by tetrad analysis and all (88 out of 88) proved to be *his4-260/his4-260*. The genotypes of all of the *his4-712/his4-712* homozygotes were also confirmed by tetrad analysis.

Independent His⁺ recombinants isolated from S1243, S1245, S1250, S1254, S1274 and S1278 were characterized by tetrad analysis (VOELKEL-MEIMAN and ROEDER 1990). Five to ten tetrads were dissected from each recombinant.

RESULTS

HOT1* promotes recombinants homozygous for a distal marker:** The effect of *HOT1* on interchromosomal recombination was examined in diploid strains carrying the *URA3* gene near the left end of one copy of chromosome *III*. A 570-bp subclone of *HOT1* (consisting of the enhancer and initiator of transcription by RNA polymerase I; VOELKEL-MEIMAN, KEIL and ROEDER 1987) was inserted just upstream of *HIS4* on one or both copies of the chromosome. The distance between *HOT1* and *URA3* is about 48 kbp (Figure 1). In these strains, recombinants that have lost the *URA3* gene can result either from interchromosomal crossing over or from gene conversion (Figure 2) and can be selected on medium containing FOA (BOEKE, LACROUTE and FINK 1984). Two sets of diploid strains were constructed, one in which *URA3* is inserted on the chromosome carrying *MATa** and one in which *URA3* is on the *MATα* chromosome.

The effect of *HOT1* on the frequency of Ura⁻ recombinants is shown in Table 2. In the absence of *HOT1*, the frequency of Ura⁻ recombinants is about 10^{-4} (S1391 and S1392). When *HOT1* is present on both chromosomes and oriented such that transcription by RNA polymerase I proceeds toward the telomere (*HOT1-t*), the frequency of Ura⁻ recombinants is increased 32–45-fold (S1394 and S1393). In strains heterozygous for *HOT1-t*, the frequency of Ura⁻ recombinants depends on whether *URA3* and *HOT1* are on the same or different copies of chromosome *III*. When *HOT1* and *URA3* are in *cis* (S1395 and S1398), the frequency of Ura⁻ recombinants is about 20-fold greater than the background level. In contrast, when *HOT1* and *URA3* are in *trans* (S1397 and S1396), Ura⁻ recombinants are increased only 2–3-fold. The stimulation of recombination observed in *HOT1* heterozygotes is not due to the heterology between the two copies of chromosome *III* because a *HOT1* fragment carrying a mutation that completely destroys *HOT1* activity has no effect on the frequency of Ura⁻ recombinants (S1282 and S1289).

The frequency of Ura⁻ recombinants is increased even when *HOT1* is oriented such that transcription by RNA polymerase I should proceed toward the centromere (*HOT1-c* in S1291, S1293 and S1295). The stimulation is much less than when *HOT1* is oppositely oriented but the difference between the *cis* and *trans* configurations of *URA3* and *HOT1* is still observed.

***HOT1*-promoted Ura⁻ recombinants are homozygous for an intervening marker:** To determine whether *HOT1*-promoted Ura⁻ recombinants are also homozygous for sequences in the interval between *HOT1* and *URA3*, the *TRP1* gene was inserted on one copy of chromosome *III* at a position about halfway between *HOT1* and *URA3* (Figure 1). Two sets of strains were constructed, one in which *TRP1* is on the same chromosome as *URA3* and another in which *URA3* and *TRP1* are on opposite homologues. Independent Ura⁻ recombinants were isolated from control strains and from *HOT1* heterozygotes in which *HOT1* and *URA3* are in *cis*. These recombinants were tested for their ability to grow in the absence of tryptophan; recombinants that were phenotypically Trp⁺ were examined by Southern blot analysis to determine whether they were homozygous or heterozygous for the *TRP1* insertion. The results are presented in Table 3.

In strain S1402, *URA3*, *TRP1* and *HOT1* are all on the same chromosome; 91% of the Ura⁻ recombinants derived from this strain are Trp⁻ and the remainder are still heterozygous for the *TRP1* insertion. In S1414, *TRP1* is on the opposite homologue to *URA3* and *HOT1*; 94% of the Ura⁻ recombinants isolated from this strain are homozygous for the *TRP1* insertion and the remainder are heterozygous for *TRP1*. Thus, most Ura⁻ recombinants are homozygous for the proximal *TRP1* marker. Sequences present at the site of the *TRP1* insertion on the *URA3*-marked parental chromosome are lost from Ura⁻ recombinants.

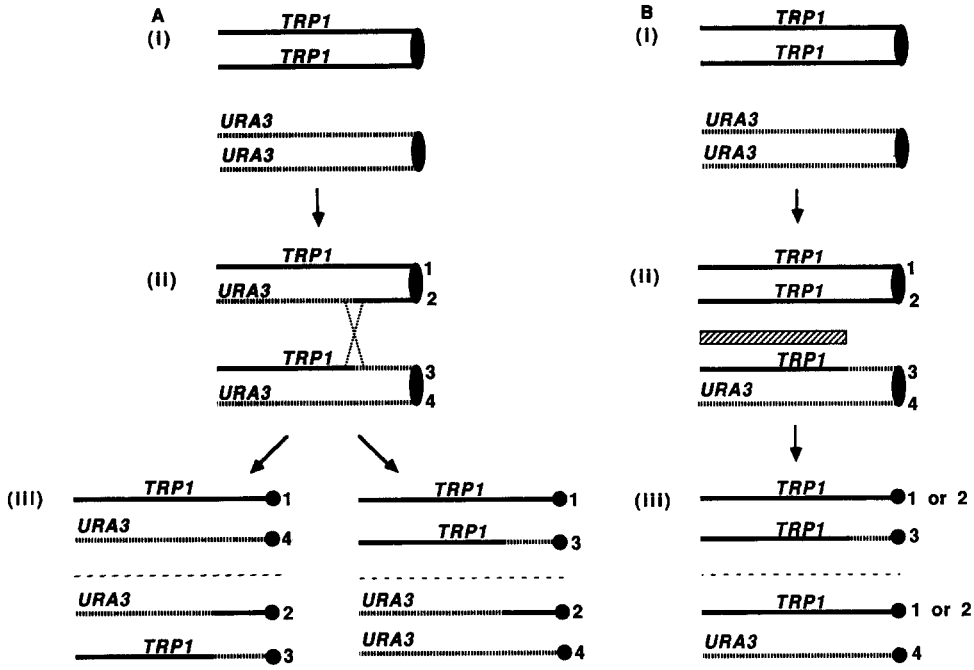

FIGURE 2.—Ura⁻ recombinants due to crossing over or gene conversion, A, Crossing over. B, Gene conversion. A (i), The parental diploid after DNA replication but before recombination. The configuration of *URA3* and *TRP1* markers indicated is that present in S1414; in the same strain, *HOT1* is present on the *URA3*-marked chromosome. Each chromosome consists of two sister chromatids represented by the solid and dashed lines. A (ii), Diploid after a crossover between *TRP1* and the centromere. The dotted lines indicate the position of the crossover. A (iii), Diploids resulting from two possible patterns of chromatid segregation. B (i), The parental diploid in G2. B (ii), Diploid after gene conversion. The hatched box indicates the region of conversion. B (iii), The daughter cells resulting from chromatid segregation.

About 35% of the Ura⁻ recombinants isolated from the control strains (S1399 and S1411) are still heterozygous at *TRP1*, as expected if a significant fraction of the events occurring in control strains initiate in sequences centromere-distal to *TRP1*. The distribution of *TRP1* homozygotes (−/−) and heterozygotes (+/−) among Ura⁻ recombinants derived from S1294, in which *HOT1* points toward the centromere, is not very different from that observed in the corresponding control (S1399). However, further characterization of the Ura⁻ Trp⁻ recombinants derived from S1399 and S1294 does reveal differences between the two strains (see below).

***HOT1*-promoted Ura⁻ recombinants result from gene conversion:** The observation that Ura⁻ recombinants are increased more when *HOT1* and *URA3* are on the same chromosome suggests that most *HOT1*-promoted Ura⁻ recombinants result from gene conversion rather than reciprocal crossing over. Reciprocal crossing over has a 50% chance (Figure 2A) of producing a Ura⁻ recombinant regardless of which chromosome initiates the exchange event; therefore, the frequency of Ura⁻ recombinants should be unaffected by the linkage between *HOT1* and *URA3*. The pronounced stimulation of Ura⁻ recombinants in strains in which *HOT1* and *URA3* are in *cis* can, however, be explained if most Ura⁻ recombinants result from gene conversion (Figure 2B) and if *HOT1* defines the chromosome on which it resides as recipient. In this case, most of the convertants arising from the *trans* configuration will be homozygous for the insertion (*URA3/URA3*) whereas most of the recombinants resulting from the *cis* configuration will be homozygous for sequences derived from the chromosome not marked with *URA3*. Although gene conversion is the simplest explanation for the results presented, there are alternative interpretations. For example, *HOT1* might stimulate reciprocal crossing over but do so only when *HOT1* and *URA3* are present on the same chromosome (*i.e.*, *HOT1* and *URA3* might somehow interact with each other to stimulate exchange).

Experiments to distinguish *HOT1*-promoted gene conversion from crossing over employed strain S1414 in which *HOT1* and *URA3* are in *cis* and *TRP1* is on the homolog (Table 3). If most *HOT1*-promoted Ura⁻ recombinants result from crossing over, then the production of a Ura⁻ (*TRP1/TRP1*) recombinant should be associated with the formation of a Trp⁻ (*URA3/URA3*) recombinant (Figure 2A). Thus, *HOT1* should promote both classes of recombinants equally. However, if most Ura⁻ recombinants result from gene conversion (in which the *HOT1*-containing chromosome is the recipient), then the formation of a Ura⁻ recombinant should not be associated with the formation of a Trp⁻ recombinant (Figure 2B) and *HOT1* should have little or no effect on the frequency of Trp⁻ recombinants in the S1414 strain background. (As shown in Table 3, sequences at the sites of the *URA3* and *TRP1* insertions remain linked in at least 90% of *HOT1*-promoted recombinants.)

Several independent cultures of S1414 were plated for single colonies on complete medium and these colonies were then replica plated to medium lacking uracil and medium lacking tryptophan to detect Ura⁻ and Trp⁻ recombinants. The data, presented in Table 4A, support the notion that *HOT1*-promoted recombinants result from gene conversion for two reasons. First, the total number of Ura⁻ recombinants re-

TABLE 2

Effect of *HOT1* on the frequency of Ura⁻ recombinants

Strain	Genotype	Ura⁻ frequency	Fold increase
S1391	$\frac{\textbf{a}}{URA3 \quad \alpha}$	0.8×10^{-4}	1.0 ×
S1394	$\frac{HOT1\text{-}t\ \textbf{a}}{URA3\ HOT1\text{-}t\ \alpha}$	25.3×10^{-4}	31.6 ×
S1395	$\frac{\textbf{a}}{URA3\ HOT1\text{-}t\ \alpha}$	16.6×10^{-4}	20.8 ×
S1397	$\frac{HOT1\text{-}t\ \textbf{a}}{URA3 \quad \alpha}$	2.1×10^{-4}	2.6 ×
S1282	$\frac{hot1\text{-}t\ \textbf{a}}{URA3 \quad \alpha}$	0.7×10^{-4}	0.9 ×
S1392	$\frac{URA3 \quad \textbf{a}}{\alpha}$	1.1×10^{-4}	1.0 ×
S1393	$\frac{URA3\ HOT1\text{-}t\ \textbf{a}}{HOT1\text{-}t\ \alpha}$	49.0×10^{-4}	44.5 ×
S1396	$\frac{URA3 \quad \textbf{a}}{HOT1\text{-}t\ \alpha}$	2.4×10^{-4}	2.2 ×
S1398	$\frac{URA3\ HOT1\text{-}t\ \textbf{a}}{\alpha}$	19.2×10^{-4}	17.5 ×
S1289	$\frac{URA3\ hot1\text{-}t\ \textbf{a}}{\alpha}$	0.9×10^{-4}	0.8 ×
S1291	$\frac{URA3\ HOT1\text{-}c\ \textbf{a}}{HOT1\text{-}c\ \alpha}$	9.0×10^{-4}	8.2 ×
S1293	$\frac{URA3 \quad \textbf{a}}{HOT1\text{-}c\ \alpha}$	1.2×10^{-4}	1.1 ×
S1295	$\frac{URA3\ HOT1\text{-}c\ \textbf{a}}{\alpha}$	2.7×10^{-4}	2.5 ×

Each of the frequencies shown is the median frequency from several independent cultures. For stains S1291, S1293 and S1295, 16 cultures were tested; 9 cultures were tested for each of the other strains. The fold increases in strains S1394, S1395, S1397 and S1282 are relative to S1391; other strains are compared to S1392. The strains listed in this Table (and their isogenic derivatives) were analyzed in at least two fluctuation tests, with similar results. For example, analysis of S1396 (in which *HOT1* and *URA3* are in *trans*) yielded a median frequency of 2.1×10^{-4} in one experiment and 2.4×10^{-4} in another; analysis of an isogenic Trp⁺ derivative of S1396 (S1413, see Figure 1 and Table 1) yielded a median frequency of 2.4×10^{-4}. An indication of the reproducibility of the results obtained with *HOT1* heterozygotes in which *HOT1* and *URA3* are in *cis* can be obtained by comparing S1395 and S1398 in this table with each other and with S1402 and S1414 in Table 3.

covered in this experiment is much greater (about 10 times) than the number of Trp⁻ recombinants. The second line of evidence comes from those cultures displaying a frequency of Ura⁻ recombinants significantly higher than the median frequency (cultures e and g in Table 4A). These represent cultures in which a recombination event occurred early in the growth of the culture and the products of this event were propagated through subsequent mitotic divisions. If this early event was a reciprocal crossover, then both Ura⁻ and Trp⁻ recombinants should be present at the same frequency (since the event would produce one of each). However, if the early event was gene conversion, then a culture which is a jackpot for Ura⁻ recombinants should not be a jackpot for Trp⁻ recombinants. In this experiment, the two cultures with a high frequency of Ura⁻ recombinants did not display a correspondingly high frequency of Trp⁻ recombinants. To detect additional examples of these early events, several independent cultures of S1414 were grown to saturation and then spot-tested to identify those with a high frequency of Ura⁻ recombinants. The cells in these cultures were plated for single colonies and then tested for their Ura and Trp phenotypes by replica plating. As shown in Table 4B, the production of a Ura⁻ recombinant was unaccompanied by the formation of a Trp⁻ recombinant in all seven of the events examined. Thus, most *HOT1*-promoted Ura⁻ recombinants result from gene conversion.

***HOT1*-promoted conversion tracts are continuous but do not always initiate at *HOT1*:** In strains S1402 and S1294, *URA3*, *TRP1* and *HOT1* are all present on the same chromosome. Ura⁻ Trp⁻ recombinants derived from these strains represent conversion tracts that cover at least the *URA3* and *TRP1* markers. To more precisely localize the right-hand endpoints of these conversion tracts, the genotypes at *HIS4* of Ura⁻ Trp⁻ recombinants were determined. The starting strain is heteroallelic at *HIS4* with the centromere-distal *his4-712* allele on the *HOT1*-containing chromosome and the *his4-260* mutation on the homolog (Figure 1). Genotypes at *HIS4* were determined as described in the Materials and Methods. The results are presented in Table 5A.

Three classes of Ura⁻ Trp⁻ recombinants are expected if conversion tracts are continuous. Convertants that have their right-hand endpoint between *TRP1* and the site of the *his4-712* mutation will still be heteroallelic at *HIS4* (*his4-712/his4-260*). Convertants with one endpoint in the interval between *his4-712* and *his4-260* will be phenotypically His⁺ and genotypically heterozygous for the *his4-260* mutation (*HIS4/his4-260*). Convertants with the right-hand endpoint on the centromere-proximal side of the site of the *his4-260* mutation will be homozygous for *his4-260*. The results presented in Table 5A indicate that 92% of the Ura⁻ Trp⁻ recombinants from the control strain (S1399) and 98% of the Ura⁻ Trp⁻ recombinants from the *HOT1*-containing strains (S1402 and S1294) can be accounted for by continuous conversion tracts. The remainder result from discontinuous conversion tracts, from chromosome loss or from conversions at *HIS4* associated with crossing over between *HIS4* and *TRP1*.

Ura⁻ derivatives of S1399, S1402 and S1294 that are still heterozygous for the *TRP1* insertion may

TABLE 3

***TRP1* genotypes of Ura⁻ recombinants**

Strain	Parental genotype	Ura⁻ frequency	Fold increase	Frequency (%) *TRP1* +/+	Frequency (%) *TRP1* +/−	Frequency (%) *TRP1* −/−
S1399	*URA3 TRP1* a / α	1.0×10^{-4}	1.0 ×	0	40	60
S1402	*URA3 TRP1 HOT1-t* a / α	14.9×10^{-4}	14.9 ×	0	9	91
S1294	*URA3 TRP1 HOT1-c* a / α	3.8×10^{-4}	3.8 ×	0	39	61
S1411	*URA3* a / *TRP1* α	1.3×10^{-4}	1.0 ×	69	31	0
S1414	*URA3* *HOT1-t* a / *TRP1* α	14.9×10^{-4}	11.5 ×	94	6	0

The recombination frequencies shown are the median frequencies derived from nine independent cultures with one exception; for S1294, 16 cultures were examined. The fold increases in S1402 and S1294 are relative to S1399; S1414 is compared to S1411. The numbers of Ura⁻ recombinants tested for their Trp phenotypes were 225 for S1399, 272 for S1402, 216 for S1294, 52 for S1411 and 50 for S1414. The numbers of Trp⁺ recombinants tested by Southern blot analysis were 90 for S1399, 10 for S1402, 28 for S1294, 52 for S1411 and 50 for S1414.

TABLE 4

Frequencies of Ura⁻ and Trp⁻ derivatives of S1414 as determined by replica plating

Culture		Ura⁻	Trp⁻	No. colonies tested
A	a	2	0	3473
	b	1	0	3100
	c	2	0	3190
	d	3	1	3170
	e	15	1	3250
	f	6	1	3260
	g	12	0	3080
	h	5	1	2940
	i	0	0	3190
B	1	35	3	3276
	2	17	0	3432
	3	14	1	2289
	4	63	2	2994
	5	26	0	2844
	6	37	0	2982
	7	7	0	2346

In the experiment shown in A, the nine independent diploids corresponding to S1414 (see MATERIALS AND METHODS) were grown to saturation, plated onto synthetic complete medium and then replica plated to medium lacking uracil and medium lacking tryptophan. In the experiment shown in B, nine cultures were grown up for each of the nine S1414 diploids; each of these was spot-tested for the frequency of Ura⁻ recombinants as described in MATERIALS AND METHODS. The seven cultures with the highest frequencies were then plated for single colonies and the resulting colonies were replica plated to detect Ura⁻ and Trp⁻ recombinants. In cultures e and g (Table 4A) and 1–7 (Table 4B), the frequency of Ura⁻ recombinants observed is significantly greater than the frequency of Trp⁻ derivatives ($P < 0.05$). See text for details.

result from events (conversions or crossovers) that are confined to *TRP1*-distal sequences. Alternatively, they may be the products of discontinuous conversion tracts in which *URA3* and one or more sites proximal to *TRP1* (but not *TRP1*) are converted. To distinguish these possibilities, Ura⁻ Trp⁺ recombinants were tested for their genotypes at *HIS4*. The vast majority are still heteroallelic (Table 5B) indicating that neither the *his4-712* nor the *his4-260* mutation has been converted. Thus, the recombination events that generate Ura⁻ Trp⁺ recombinants are largely confined to sequences distal to *TRP1*.

***HOT1*-promoted conversion tracts frequently include sequences to both sides of *HOT1*:** S1402 and S1294 derivatives that are Ura⁻ Trp⁻ and homozygous for *his4-260* could result from conversion tracts whose right-hand endpoints lie in the interval between *his4-260* and *HOT1* or from conversion tracts that extend further to the right. These recombinants were examined by Southern blot analysis to determine whether or not they were still heterozygous for the *HOT1* insertion. In addition, the diploids were sporulated and tetrads were analyzed in order to determine the genotype at the *LEU2* gene, located about halfway between *HIS4* and the centromere (Figure 1). The results are presented in Table 6.

About 6% of the *his4-260* homozygotes derived from S1402 and 3% from S1294 are still heterozygous for *HOT1* indicating that the conversion tract has one endpoint in the interval between *HOT1* and the *his4-260* mutation. More than 90% do not carry *HOT1* on either chromosome, indicating a continuous conversion tract that includes *HOT1*. About half of these are still heterozygous at *LEU2* and the remainder are homozygous for the wild-type *LEU2* gene. The latter class represent continuous conversion tracts extending

TABLE 5

***HIS4* genotypes of Ura⁻ recombinants**

	Strain	Parental genotype	Alleles recovered (%)							No. tested
			712/260	+/260	260/260	712/712	260	+/+	712/+	
A Trp⁻	S1399	*URA3 TRP1 his4-712* **a** / *his4-260* α	45.2	2.2	44.4	1.5	5.2	0.7	0.7	135
	S1402	*URA3 TRP1 his4-712 HOT1-t* **a** / *his4-260* α	41.1	11.4	45.6	0.8	0.0	0.0	1.1	248
	S1294	*URA3 TRP1 his4-712 HOT1-c* **a** / *his4-260* α	19.1	3.1	76.3	0.8	0.8	0.0	0.0	131
B Trp⁺	S1399	*URA3 TRP1 his4-712* **a** / *his4-260* α	100.0	0.0	0.0	0.0	0.0	0.0	0.0	90
	S1402	*URA3 TRP1 his4-712 HOT1-t* **a** / *his4-260* α	91.6	0.0	8.3	0.0	0.0	0.0	0.0	24
	S1294	*URA3 TRP1 his4-712 HOT1-c* **a** / *his4-260* α	100.0	0.0	0.0	0.0	0.0	0.0	0.0	85

The percentages of Ura⁻ Trp⁻ and Ura⁻ Trp⁺ recombinants with different genotypes at *HIS4* are given. Derivatives described as *his4-260* were identified as $2n - 1$ strains based on several criteria: (1) they mate efficiently with *MAT***a** testers, (2) they fail to sporulate and (3) when transformed with a *MAT***a** plasmid, sporulated and dissected, they produce 2 viable:2 dead spores per tetrad.

TABLE 6

***HOT1* and *LEU2* genotypes of *his4-260/his4-260* recombinants**

Recombinant genotype	Frequency (%)	
	S1402 *URA3 TRP1 his4-712 HOT1-t leu2* **a** / *his4-260* *LEU2* α	S1294 *URA3 TRP1 his4-712 HOT1-c leu2* **a** / *his4-260* *LEU2* α
his4-260 HOT1 leu2 / *his4-260 LEU2*	5.9	2.6
his4-260 leu2 / *his4-260 LEU2*	44.1	42.1
his4-260 LEU2 / *his4-260 LEU2*	47.1	55.2
his4-260 HOT1 leu2 / *his4-260 HOT1 LEU2*	2.9	0.0

The percentages of Ura⁻ Trp⁻ *his4-260/his4-260* recombinants with different genotypes at *HOT1* and *LEU2* are given. The numbers of recombinants analyzed by tetrad dissection and Southern blot hybridization were 34 for S1402 and 38 for S1294.

all the way from the centromere-proximal side of *LEU2* through *URA3*. A small number (3%) of the S1402 recombinants represent discontinuous conversion tracts in which *HOT1* was converted in the opposite direction to *URA3*, *TRP1* and *HIS4* or conversion at *HOT1* was followed by crossing over.

Ura⁻ Trp⁻ recombinants that are *his4-712/his4-260* or *HIS4/his4-260* in genotype can be explained by long conversion tracts that have their right-hand endpoints in the *TRP1-his4-712* or the *his4-712-his4-260* interval, respectively. If this explanation is correct, then these recombinants should show no signs of conversion at sites proximal to *his4-260*. To explore this possibility, a number of *his4-712/his4-260* and *HIS4/his4-260* recombinants derived from S1402 and S1294 were analyzed by Southern blot analysis to determine their genotypes at *HOT1* and by tetrad dissection to determine their genotypes at *LEU2*. All but 2% (1 out of 52) were still heterozygous at *HOT1* and all but 4% (2 out of 45) were still heterozygous for the *leu2* mutation.

Summary of Ura⁻ data: A summary of the analysis of Ura⁻ recombinants derived from the control strain, S1399, and the *HOT1* heterozygotes, S1402 and S1294, is shown in Figure 3. As indicated in Figure 2, Ura⁻ recombinants can result from either gene conversion or crossing over. The fact that *HOT1* stimulates recombination 10 times more when *HOT1* and *URA3* are in *cis vs.* in *trans* suggests that about 90% of the Ura⁻ recombinants derived from S1402

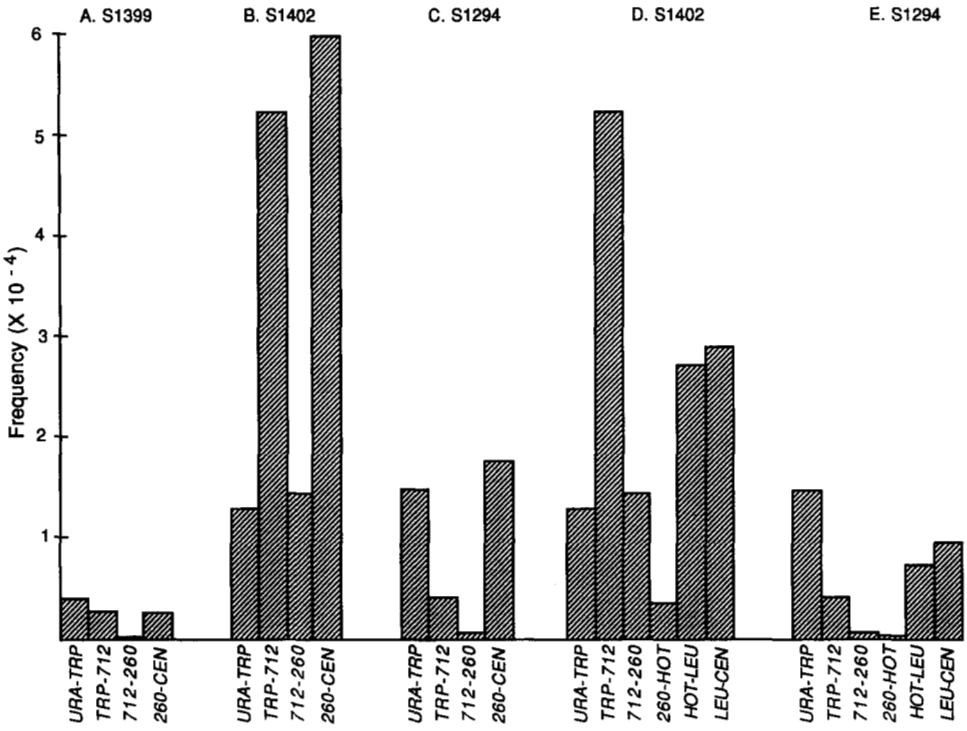

FIGURE 3.—Distributions of recombination events. The histograms indicate the distributions of right-hand conversion endpoints or crossover sites for Ura$^-$ recombinants derived from S1399, S1402 and S1294. A, B and C show the frequencies of events in the *URA3-TRP1* (*URA-TRP*), *TRP1-his4-712* (*TRP-712*), *his4-712-his4-260* (*712-260*) and *his4-260*-centromere (*260-CEN*) intervals. In D and E, the *his4-260-CEN3* interval is further subdivided into the *his4-260-HOT1* (*260-HOT*), *HOT1-LEU2* (*HOT-LEU*) and *LEU2-CEN3* (*LEU-CEN*) intervals. Values were calculated from the information in Tables 3, 5 and 6 and the text. For example, the frequency of events occurring in the *HOT1-LEU2* interval in S1294 is the percent of Ura$^-$ recombinants that are Trp$^-$ (61%) multiplied by the percent of Ura$^-$ Trp$^-$ recombinants that are homozygous for *his4-260* (76.3%) multiplied by the percent of *his-260* homozygotes that are homozygous at *HOT1* (−/−) but still heterozygous at *LEU2* (42.1%) multiplied by the frequency of Ura$^-$ recombinants (3.8×10^{-4}).

result from gene conversion. By the same reasoning, half to three-quarters of the Ura$^-$ recombinants derived from S1294 are due to conversion. It is unknown what fraction of the Ura$^-$ recombinants derived from the control strain are due to conversion versus reciprocal exchange. For those Ura$^-$ recombinants that result from gene conversion, the left end of the conversion tract must lie to the left of *URA3*. Figure 3 shows the distribution of right-hand conversion endpoints (for Ura$^-$ recombinants due to gene conversion) or of crossovers (for Ura$^-$ recombinants due to crossing over).

In the control strain, events are more or less equally distributed among the *URA3-TRP1*, *TRP1-his4-712* and *his4-260-CEN3* intervals. In S1402, containing *HOT1-t*, the frequencies of events in all intervals are increased and there is a shift in the distribution of events toward more centromere-proximal intervals (Figure 3). In S1294, containing *HOT1-c*, the frequencies of events in all intervals are increased compared to the control but the shift in distribution is not as pronounced as that observed for S1402.

Long conversion tracts among His$^+$ recombinants: We have previously examined the effect of *HOT1* on the production of His$^+$ prototrophs in strains carrying *his4* heteroalleles (*his4-712/his4-260*) and a distal, heterozygous *URA3* marker (VOELKEL-MEIMAN and ROEDER 1990). The frequency of His$^+$ recombinants was increased about 9-fold when *HOT1* was inserted just upstream of *HIS4* on both chromosomes and about 7-fold when *HOT1* was present only on the *his4-712* chromosome. About 70% of the His$^+$ recombinants were still heterozygous for *URA3* indicating that the distal marker was not converted. It should be noted that the frequency of His$^+$ recombinants (10^{-4}) in *HOT1*-containing strains is 20–50-fold lower than the frequency of Ura$^-$ recombinants ($2–5 \times 10^{-3}$). Thus, His$^+$ Ura$^-$ recombinants represent only a small fraction of total Ura$^-$ recombinants.

Approximately 30% of His$^+$ recombinants were homozygous for the distal marker (either *URA3/URA3* or Ura$^-$). These recombinants could result either from gene conversion at *HIS4* accompanied by crossing over or from long conversion tracts extending from *HIS4* through *URA3* (Figure 4). To distinguish these possibilities, independent His$^+$ recombinants, unselected for their genotype at *URA3*, were analyzed by tetrad dissection. When gene conversion at *HIS4* is accompanied by a crossover, the His$^+$ gene will segregate into a cell that is homozygous for the distal

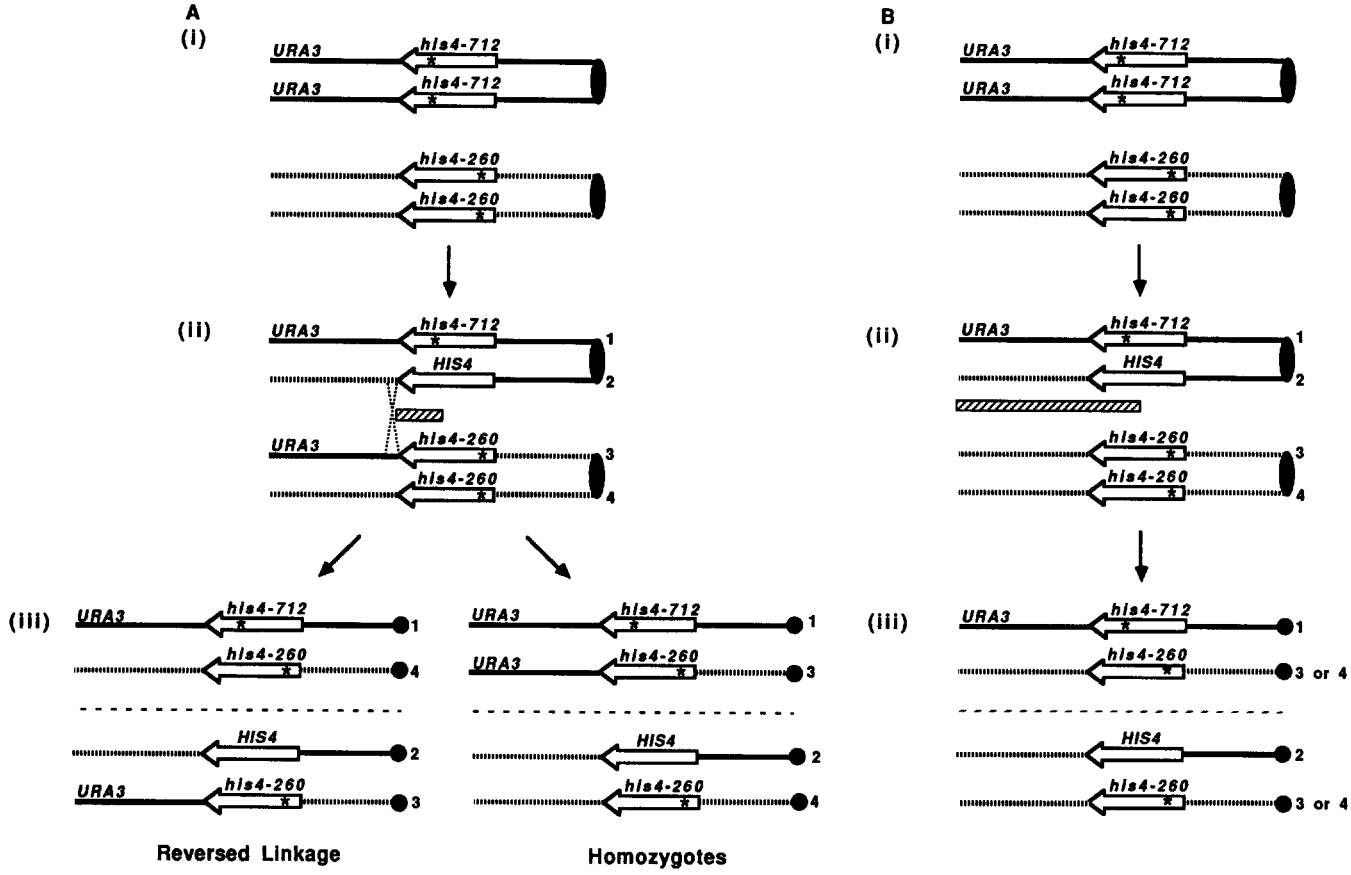

FIGURE 4.—His$^+$ Ura$^-$ recombinants resulting from crossing over or gene conversion. A, Conversion at *HIS4* accompanied by crossing over. B, Coconversion of *his4-712* and *URA3*. A (i), The parental diploid in G_2. A (ii), Diploid after conversion of *his4-712* accompanied by a crossover between *his4-712* and *URA3*. A (iii), Diploids resulting from two possible patterns of chromatid segregation. B (i), Parental diploid. B (ii), Diploid after gene conversion spanning the *his4-712* and *URA3* markers. B (iii), Daughter cells resulting from chromatid segregation. The recombination event diagrammed in A represents only one of several possible types of gene conversion associated with crossing over. Both conversion of *his4-712* accompanied by a crossover between *HIS4* and *URA3* (diagrammed) as well as conversion of *his4-260* accompanied by a crossover between *HIS4* and *LEU2* will give rise to His$^+$ recombinants that are homozygous for the distal marker originally present on the *his4-260* chromosome (*U2/U2* in Table 7). These are the two major classes of recombinants expected if conversion tracts are continuous and if crossing over occurs at one end of the conversion tract. His$^+$ recombinants that are homozygous for the distal marker on the *his4-712* chromosome (*U1/U1* in Table 7) can result from conversion of *his4-260* accompanied by a crossover in the *HIS4* to *URA3* interval or from conversion of *his4-712* accompanied by a crossover in the *HIS4* to *LEU2* interval. These recombinants presumably result from discontinuous conversion tracts in which the *his4* allele that is converted is separated from the crossover by an unconverted *his4* mutation. The open arrow indicates the direction of *HIS4* transcription; the asterisks indicate *his4* mutations. Other symbols are as in Figure 2. Drawing is not to scale.

marker 50% of the time (Figure 4A). The other half of the time, the His$^+$ gene will segregate into a cell which is still heterozygous for *URA3* but displays reversed (nonparental) linkage between the unconverted *his4* allele and either *URA3* or *LEU2* (depending on whether the crossover occurred in the *URA3-HIS4* or *HIS4-LEU2* interval). Thus, if most His$^+$ *URA3/URA3* and His$^+$ Ura$^-$ recombinants result from conversion associated with crossing over, the number of His$^+$ recombinants displaying reversed linkage should be equal in number to the sum of the two classes of homozygotes. To determine whether this is the case, His$^+$ recombinants were sporulated and tetrads were dissected and analyzed. The *his4* alleles present in haploid His$^-$ spore clones were determined by allele tests (VOELKEL-MEIMAN and ROEDER 1990). The linkage between *URA3* and *HIS4* and between *HIS4* and *LEU2* was determined; from this information, the linkage between *URA3* and *LEU2* was deduced.

Three sets of diploid strains were examined: (1) control strains lacking *HOT1*, (2) *HOT1* homozygotes and (3) strains carrying *HOT1* only on the chromosome marked with *his4-712* and *leu2*. The results are summarized in Table 7. In none of the strains was a 1:1 ratio of homozygotes to reversed linkages observed. The ratio was 2.1:1 (15:7) in the control strain, 1.9:1 (37:19) in the *HOT1* homozygote and 2.4:1 (29:12) in the *HOT1* heterozygote. In both of the *HOT1*-containing strains, the results are significantly different from those expected for a 1:1 ratio ($P < 0.01$). Although the control strain displays a 2.1:1 ratio of homozygotes to reversed linkages, this ratio is not significantly different from a 1:1 ratio ($P < 0.1$) due to the low frequency of recombinants of both types. The excess of homozygotes to reversed linkages

TABLE 7

Genotypes of His$^+$ recombinants

		S1243 and S1245	S1250 and S1254	S1274 and S1278
		U1 712 leu2 / *U2 260 LEU2*	*U1 712 HOT1 leu2* / *U2 260 HOT1 LEU2*	*U1 712 HOT1 leu2* / *U2 260 LEU2*
Parental linkage	*U1 leu2* / *U2 LEU2*	85	62	69
Reversed linkage	*U2 leu2* / *U1 LEU2*	7	19	12
U1 homozygotes	*U1 leu2* / *U1 LEU2*	4	11	8
U2 homozygotes	*U2 leu2* / *U2 LEU2*	11	26	21
Other		6	2	3
Total homozygotes (U1 and U2)		15	37	29
Total recombinants tested		113	120	113

U1 and *U2* refer to the distal marker (*URA3* or no *URA3*) present on the *his4-712 leu2* chromosome and the *his4-260 LEU2* chromosome, respectively. For all three kinds of strains (controls, *HOT1* homozygotes and *HOT1* heterozygotes), two sets of diploids were constructed and analyzed, one with *URA3* on the *his4-712* chromosome and one with *URA3* on the *his4-260* chromosome (see Table 1). For example, *URA3* is linked to *his4-260* in S1243 and to *his4-712* in S1245. Thus, a *U1/U1* homozygote derived from S1243 is Ura$^-$ whereas a *U1/U1* homozygote derived from S1245 is *URA3/URA3*. The vast majority (>90%) of His$^+$ recombinants carried a *HIS4*$^+$ gene on one chromosome and either *his4-712* or *his4-260* on the homolog; a small number were heterozygous for the double mutation (*his4-260,712*) or homozygous *HIS4*$^+$ (VOELKEL-MEIMAN and ROEDER 1990).

suggests that, at least in *HOT1*-containing strains, some recombinants may result from gene conversion both at *HIS4* and *URA3*.

A long, continuous conversion tract will generate a His$^+$ recombinant that is homozygous for the distal marker if the *his4-712* chromosome is the recipient and if the conversion event initiates in the interval between *his4-712* and *his4-260* and extends to the left of the *URA3* gene. The resulting His$^+$ recombinant will be heterozygous for the *his4-260* mutation and homozygous for the distal marker originally present on the chromosome marked with *his4-260* (*U2*). As shown in Table 7, almost three-quarters of the homozygous recombinants are homozygous for *U2*; the majority of these (about 90%) are heterozygous for *his4-260* (data not shown). Thus, the majority of the recombinants have the phenotype expected to result from coconversion.

The data in Table 7 demonstrate that, in the presence of *HOT1*, 52% (*HOT1* homozygotes) to 61% (*HOT1* heterozygotes) of the His$^+$ recombinants result from conversions at *HIS4* (but not *URA3* or *LEU2*) unassociated with crossing over. Another 32% to 21% result from gene conversion associated with reciprocal crossing over [2 (reversed linkages)/total × 100] and 15% result from conversion of both *HIS4* and *URA3* [(homozygotes − reversed linkages)/total × 100]. In the control strain, 75% of the His$^+$ recombinants are due to conversion unaccompanied by crossing over; 14% are due to conversion associated with crossing over and 7% are due to coconversion. *HOT1* increases the frequencies of all three classes of events but it stimulates conversions associated with crossing over and coconversions even more than it stimulates short conversion tracts that are unaccompanied by crossovers.

DISCUSSION

***HOT1* promotes long, continuous conversion tracts:** We have examined the effect of *HOT1* in diploid strains carrying a heterozygous *URA3* marker on the left arm of chromosome *III*. We found that the frequency of Ura$^-$ recombinants in *HOT1* heterozygotes is severely affected by the linkage between *HOT1* and *URA3*. Ura$^-$ recombinants are increased dramatically only in strains in which *HOT1* and *URA3* are in *cis*. Furthermore, the stimulation of Ura$^-$ recombinants observed when *HOT1* and *URA3* are present on the same chromosome is unaccompanied by a corresponding increase in the frequency of recombinants homozygous for sequences on the *URA3*-marked homolog. These results indicate that most *HOT1*-promoted Ura$^-$ recombinants result from gene conversion and that sequences on the *HOT1*-containing chromosome are preferentially converted, consistent with our previous studies of gene conversion at *HIS4* (VOELKEL-MEIMAN and ROEDER 1990).

HOT1-promoted conversion tracts were examined in strains carrying multiple markers on the left arm of chromosome *III*. Most of the conversion tracts giving rise to Ura$^-$ recombinants were at least 30 kbp in length and many were longer than 77 kbp; the

average conversion tract was 43 kbp long in S1402 (*HOT1-t*) and 36 kbp in S1294 (*HOT1-c*). [Note that the average length calculated is a minimum value. For example, conversion tracts that include *URA3* and *TRP1* but not any of the other scorable markers are assumed to be 30 kbp in length—the size of the *URA3* insertion (1.2 kbp) plus the distance between *URA3* and *TRP1* (28 kbp) plus the size of the *TRP1* insertion (0.8 kbp). In reality, the region of conversion might have extended almost to the site of the *his4-712* mutation in which case the tract would be 48 kbp long.] The fraction of events that can be accounted for by continuous conversion tracts based on the data available is 98% for S1294 (*HOT1-c*) and 93% for S1402 (*HOT1-t*). The frequency of discontinuous conversion tracts may be slightly underestimated since recombinants were not tested for conversion of all markers proximal to a locus which did not undergo conversion.

It could be argued that most *HOT1*-promoted conversion tracts are much shorter than 40 kbp but that the selection imposed (which demands loss of a marker far from the presumed initiation site) leads to an artifactual inflation of conversion tract length. If most *HOT1*-promoted conversion tracts initiate near *HOT1* but extend for a distance of only one or a few kbp, then a marker inserted near *HOT1* would be expected to undergo a higher frequency of conversion than a distant marker. In fact, strains in which *URA3* was inserted less than 3 kbp downstream of *HOT1-t* produced 2–3-fold fewer Ura$^-$ recombinants than strains carrying *URA3* at the more distal location (data not shown). This is the result expected if most *HOT1*-promoted conversion tracts extend all the way to the end of the chromosome and if half of the conversion events initiate in or proximal to the *HIS4* gene and the other half are confined to *HIS4*-distal sequences (as indicated by the analysis of S1402 recombinants).

Other investigators have measured conversion tract lengths during mitotic interchromosomal recombination. JUDD and PETES (1988) selected Ura$^-$ convertants from a diploid strain heterozygous for a *ura3* mutation and then examined these recombinants for conversion of flanking restriction sites that were heterozygous in the parent. In about half of the Ura$^-$ recombinants examined, conversion tracts were shorter than 4 kbp; the other half were longer but their lengths could not be accurately determined.

Diploid strains heterozygous for an auxotrophic mutation (+/−) have also been used to screen for sectored colonies that are prototrophic on one side and auxotrophic on the other. These sectored colonies can result from reciprocal recombination, in which case the prototrophic half of the colony is homozygous (+/+), or from nonreciprocal recombination, in which case the prototrophic sector is heterozygous (+/−). When spontaneous sectored colonies were examined, the majority resulted from nonreciprocal recombination; furthermore, gene conversion at loci linked to the marker used to detect recombination was frequently observed (JOHNSTON 1971; BRUSCHI and ESPOSITO 1983). For example, JOHNSTON (1971) found that 10 of 18 colonies sectored for *ade8* were due to nonreciprocal recombination; half of these also showed conversion of the *TRP4* gene located about 40 cM centromere-proximal to *ADE8*.

Mechanism of *HOT1*-promoted gene conversion: We previously considered two models to account for the fact that a *HOT1*-containing chromosome preferentially received information during gene conversion. In one of these models, *HOT1*-promoted gene conversion is assumed to result from asymmetric strand transfer (MESELSON and RADDING 1975) with the *HOT1*-containing chromosome acting as recipient (Figure 5, A and B). According to this model, *HOT1* stimulates recombination by rendering the adjacent sequences more susceptible to strand invasion.

In order for the asymmetric strand exchange model to explain the long conversion tracts described here, the region of heteroduplex DNA must frequently extend all the way from *LEU2* through *URA3*. Furthermore, all of the markers included in the heteroduplex must be repaired in the same direction. This could result if the mismatches were left unrepaired and then resolved by DNA replication (Figure 5A) or if the mismatch repair system corrected all mismatches in the same direction (Figure 5B). Both of these modes of repair seem unlikely for the following reasons. First, mismatch repair in mitosis is very efficient. ESPOSITO and WAGSTAFF (1981) estimated a minimum efficiency of 60–70% for mismatched base pairs at the *TRP1*, *LEU1* and *HIS1* loci. In studies of repair of heteroduplex plasmid DNA transformed into yeast (BISHOP and KOLODNER 1986), single bp mismatches, as well as 8-bp and 12-bp insertion mismatches, were repaired in 73–98% of the transformants analyzed. Unfortunately, there is no information bearing on the efficiency of repair of large insertion mismatches such as those that would be created at *URA3*, *TRP1* and *HOT1*; nevertheless, a failure of repair at six mismatched sites (*URA3*, *TRP1*, *his4-712*, *his4-260*, *HOT1* and *LEU2*), three of which represent single bp mismatches, seems unlikely. In order for all mismatches to be corrected in the same direction by the mismatch repair system, they would presumably have to be included in the same repair tract. Studies of heteroduplex repair during transformation indicate that repair tracts are frequently shorter than 1 kb (BISHOP and KOLODNER 1986). During mismatch correction *in vitro* in extracts of mitotic cells, excision-resynthesis tracts are only 10–20 nucleotides long (MUSTER-NASSAL and KOLODNER 1986).

A model that better explains *HOT1*-promoted gene

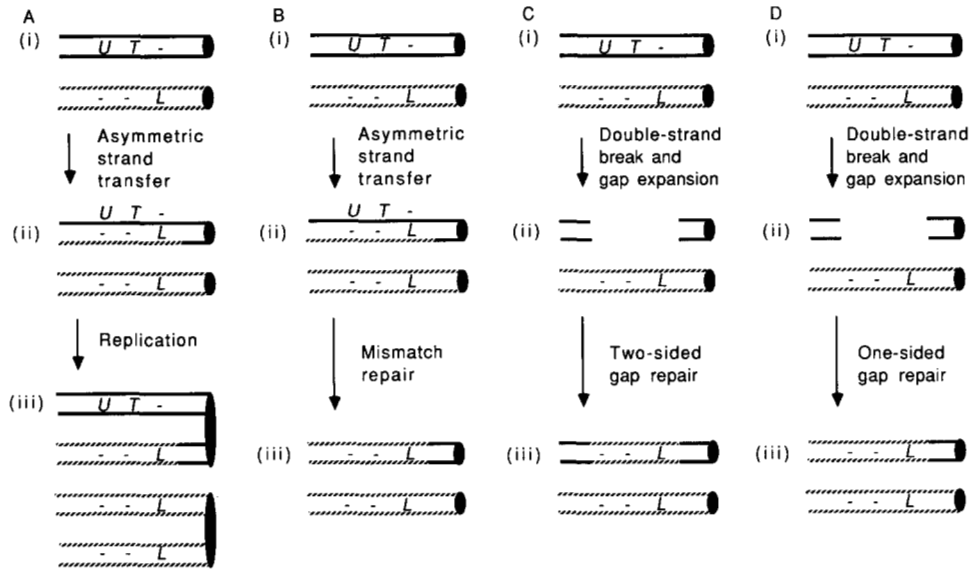

FIGURE 5.—Models for *HOT1*-promoted gene conversion. A, Asymmetric strand exchange followed by repair by replication. B, Asymmetric strand exchange followed by mismatch repair. C, Double-strand breakage followed by two-sided repair. D, Double-strand breakage followed by one-sided repair. A (i), Parental diploid in G_1. A (ii), Diploid after asymmetric strand exchange. A (iii), Diploid after DNA replication. B (i), Parental diploid. B (ii), Diploid after asymmetric strand exchange. B (iii), Diploid after mismatch repair. C (i), Parental diploid. C (ii), Diploid after double-strand breakage and gap expansion. C (iii), Diploid after two-sided gap repair. D (i), Parental diploid. D (ii), Diploid after double-strand breakage and gap expansion. D (III), Diploid after one-sided gap repair. The model in A and B was first proposed by MESELSON and RADDING (1975); the model in C is the double-strand-break repair model (SZOSTAK *et al.* 1983) and D is a modification of this model. See text for additional information. Each line represents a single strand of a duplex DNA molecule; the strands from one duplex are indicated by solid lines and the strands from the other are hatched. *U*, *T* and *L* indicate the *URA3*, *TRP1* and *LEU2* genes, respectively; the corresponding − signs indicate the absence of the *URA3* or *TRP1* gene or the recessive *leu2* mutation. For homoduplexes, the genotype is indicated between the two strands; for heteroduplexes, the genotype of each strand is given. In the examples shown, the region of asymmetric strand exchange (A and B) and the double-strand gap (C and D) include the *URA3*, *TRP1* and *LEU2* markers. For simplicity, only two recombining DNA molecules are shown even though recombination may occur in G_2, when four chromatids are present, as diagrammed in Figures 2 and 3.

conversion events proposes that *HOT1* stimulates the formation of a double-strand break in the region of *HOT1*-promoted transcription (Figure 5, C and D). This model can account for the long, continuous conversion tracts described in this paper if it is assumed that breakage is frequently followed by extensive exonucleolytic digestion. The gap generated could then be repaired (SZOSTAK *et al.* 1983) if the free ends created on both sides of the break invade the homolog and initiate repair synthesis (Figure 5C). An alternative to gap repair is a "break-and-replicate" pathway of recombination analogous to that used by bacteriophage T4 (MOSIG 1987). According to this version of the model, only the centromere-containing chromosomal fragment invades the homolog and primes replication (Figure 5D). Sequences distal to the break might be enzymatically degraded; if not degraded, then the fragment would be lost at mitosis due to the absence of a centromere.

Ura$^-$ derivatives that are mating-competent might be expected to result either from chromosome loss or from conversion tracts that include the *MAT* locus. However, only one example of a mating-competent segregant was detected among over 500 independent *HOT1*-promoted Ura$^-$ recombinants analyzed (data not shown) and this segregant resulted from chromosome loss. If most Ura$^-$ recombinants result from double-strand-break-induced recombination, then the low frequency of chromosome loss indicates that double-strand break repair is very efficient. Furthermore, the data suggest that the region of exonucleolytic digestion (and subsequent repair) rarely includes the *MAT* locus. This could be due to the distance involved (about 150 kbp from *HOT1* to *MAT*) or to the presence of a specific sequence (such as the centromere) that might inhibit gap expansion.

Do *HOT1*-promoted recombination events initiate in *HOT1*-transcribed sequences? The distributions of recombination events in *HOT1*-containing strains suggest that not all *HOT1*-promoted recombination events initiate in sequences undergoing transcription by RNA polymerase I. Previous studies have suggested that transcription initiating in *HOT1* can extend for a distance of about 12 kbp (STEWART and ROEDER 1989). Thus, most events are expected to initiate in the *TRP1-HOT1* interval in S1402 (*HOT1-t*) and between *HOT1* and *LEU2* in S1294 (*HOT1-c*). Conversion tracts that have their right-hand endpoints proximal to the presumed region of initiation can be accounted for by double-strand breakage followed by

exonucleolytic digestion. However, conversions whose right-hand endpoints are distal to the region of transcription are more difficult to explain. Nevertheless, these events are stimulated by *HOT1*. For example, events in the *URA3-TRP1* interval are increased 3–4-fold for both S1402 and S1294. In S1402, these distal events can be explained by proposing that some *HOT1*-promoted transcripts are significantly longer than 12 kbp and therefore extend past *TRP1*. This explanation does not, however, apply to S1294.

There are several possible explanations for the ability of *HOT1* to stimulate events confined to sequences distal to the region of *HOT1*-promoted transcription. First, *HOT1*-promoted transcription might somehow interfere with the spontaneous (*i.e.*, not *HOT1*-promoted) recombination events that would normally occur in the region of *HOT1*-promoted transcription. This inhibition might result in a redistribution of the spontaneous events such that these are increased in frequency in sequences distant from the site of *HOT1* insertion. Alternatively, *HOT1* may be able to act at a distance to trigger the initiation of events outside the region of transcription. For example, an enzyme may enter the DNA in the region of *HOT1*-promoted transcription and then travel some distance before introducing a break. The RecBC enzyme of *Escherichia coli* is an example of an enzyme that travels some distance before cutting (PONTICELLI *et al.* 1985); this enzyme requires a free duplex end in order to enter a DNA molecule (STAHL *et al.* 1983). Another possibility is that *HOT1* promotes the formation of a nick (in the region of transcription) which is expanded to a single-strand gap; the exposed single strand could subsequently be cleaved by an endonuclease to generate a double-strand break. If the second cut occurs at some distance from the first, then the double-strand break could be distant from the region of *HOT1*-promoted transcription. Models similar to these have been proposed to explain the ability of HO-induced breaks to stimulated recombination at a distance (RUDIN and HABER 1988; NICKOLOFF *et al.* 1989; RAY, MACHLIN and STAHL 1989).

Another puzzling observation is that *HOT1-t* stimulates the formation of Ura$^-$ recombinants significantly more than *HOT1-c*. The simplest explanation for this result is that *HOT1-c* is less active than *HOT1-t* due to a context effect. For example, the DNA that lies proximal to *HOT1* might fortuitously contain sequences that terminate transcription by RNA polymerase I. In this case, the region of *HOT1*-promoted transcription might be shorter in strains containing *HOT1-c* than *HOT1-t*. Alternatively, when *HOT1* is oriented toward the centromere, the region of transcription may be so far from *URA3* that conversion events "initiating" in the transcribed region rarely include *URA3*. If *HOT1* promotes transcription over a distance of 12 kbp, then *HOT1-t* transcribes sequences 34–46 kbp from *URA3* and *HOT1-c* transcribes sequences 46–58 kbp from *URA3*. To account for the 4-fold difference in recombination frequency between S1402 and S1294, it is necessary to argue that *HOT1* promotes the conversion of a marker 46–58 kbp away 4-fold less often than it promotes conversion of a marker 34–46 kbp away. Given the extreme lengths of the conversion tracts observed, it seems unlikely that the frequency of events decreases so abruptly. A third possibility is that *HOT1-c* promotes events with the same efficiency as *HOT1-t* but these conversion tracts have a directionality such that they often include sequences downstream (to the right of *HOT1-c*) and less infrequently sequences upstream. Since the strains used in this study carry no selectable markers to the right of *HOT1*, we have been unable to explore this possibility.

Coincident conversion of *HIS4* and *URA3*: When gene conversion at *HIS4* is accompanied by reciprocal crossing over in the G_2 stage of the cell cycle (Figure 4A), half of the resulting His$^+$ recombinants are expected to display a nonparental configuration of markers flanking *HIS4* (reversed linkage) and half should be homozygous for a distal marker that was originally heterozygous. HABER and HEARN (1985) observed a 1:1 ratio of reversed linkages to homozygotes in their studies of gene conversion at *HIS4* using strains and selections very similar to those described here.

Three factors might lead to an alteration in the expected 1:1 ratio of reversed linkages to homozygotes. First, if any crossovers occur in G_1, then reversed linkages will exceed homozygotes because crossovers in G_1 generate only recombinants displaying reversed linkage. In fact, ROMAN (1980) observed a 2-fold excess of reversed linkages in his studies of X-ray induced recombination at *ADE6*. A second factor which can affect the ratio is if the recombinant chromatids segregate nonrandomly at mitosis. In studies of mitotic recombination in Drosophila, PIMPINELLI and RIPOLL (1986) observed a 2-fold bias in favor of the recombinant chromosomes segregating to opposite poles. In the experiments reported here, such a bias would result in a 2-fold excess of homozygotes to reverse linkages, which is precisely the result observed. An alternative explanation for the excess of reverse linkages to homozygotes is that recombinants homozygous for the distal marker result not from crossing over but from nonreciprocal recombination. If random chromatid segregation is assumed (as it will be throughout the remainder of this discussion), then the observed 2:1 ratio of homozygotes to reversed linkages suggests that about half of the homozygotes result from crossing over and half from gene conversion. Note that this is a minimum estimate of the frequency of coincident conversions since it

assumes that all crossovers occur in G_2. If some crossovers occur in G_1 (leading to an increase in the number of reversed linkages), then the number of homozygotes resulting from conversion must be greater.

Recombinants that have undergone conversion both at *HIS4* and *URA3* could result either from coconversion (both markers included in the same conversion tract) or from coincident (but separate) conversion events. The observation that the majority of His^+ recombinants that are homozygous for the distal marker have the genotype expected for long continuous conversion tracts (*U2 HIS4/U2 his4-260*; see Table 7 and RESULTS) is consistent with the hypothesis that *HIS4* and *URA3* are coconverted. However, this result is inconclusive because the same class of recombinants is expected to be the predominant product of conversions associated with crossing over (see Figure 4 and legend). In the absence of suitable markers in the interval between *HIS4* and *URA3*, it is impossible to determine whether simultaneous conversions of *HIS4* and *URA3* represent continuous conversion tracts or coincident conversions.

It should be noted that very different kinds of *HOT1*-promoted recombinants are recovered depending on whether His^+ or Ura^- recombinants are selected. The majority (80–85%) of His^+ recombinants result from relatively short conversion tracts (that include one of the *his4* alleles but not the other *his4* mutation and not *URA3* or *LEU2*) with or without an associated crossover. Only 15% are due to coincident conversions (possibly coconversions). In contrast, almost all Ura^- recombinants result from long, continuous conversion tracts. These results can be explained by the models shown in Figure 5, C and D, if it assumed that most His^+ recombinants result from limited gap expansion whereas Ura^- recombinants result from more extensive exonucleolytic digestion. Most His^+ and perhaps some Ura^- recombinants result when sequences on both sides of the break invade the homolog and promote repair synthesis (Figure 5C). Many Ura^- recombinants and perhaps a small fraction of His^+ recombinants result from one-ended repair synthesis (Figure 5D). While this is an economical interpretation of our data, the possibility that His^+ and Ura^- recombinants result from fundamentally different mechanisms cannot be ruled out. For example, Ura^- recombinants might result from a break-and-replicate pathway of recombination whereas most His^+ recombinants might result from asymmetric strand exchange. As noted above, His^+ recombinants are 20–50-fold less frequent than Ura^- recombinants.

Other studies of intragenic recombination in heteroallelic diploids have provided evidence for coincident conversions of distant markers. ESPOSITO and coworkers used diploid strains carrying heteroalleles at the *TRP5* locus on chromosome VII and a distal heterozygous *ade5* mutation (ESPOSITO 1978; GOLIN and ESPOSITO 1983; BRUSCHI and ESPOSITO 1983). They selected Trp^+ colonies that were sectored for the *ade5* mutation. The majority of these recombinants resulted from gene conversion at *TRP5* accompanied by crossing over between *TRP5* and *ADE5* but about 15% resulted from conversion both at *TRP5* and *ADE5*. In slightly less than half of these "coconvertants," markers in the interval between *TRP5* and *ADE5* (*cyh2* and *met13*) were converted in the same direction as *ade5*. In the remainder, one or both of the intervening markers went unconverted, was converted in the opposite direction to *ade5* or displayed 4:0 (instead of 3:1) segregation.

In studies of diploids heteroallelic at the *LEU1* and *TRP5* loci (*TRP5* is distal to *LEU1* on chromosome *VII*), GOLIN and ESPOSITO (1984) observed that coincident (Leu^+ Trp^+) convertants arose 1200 times more frequently than expected for independent events. Furthermore, an insertion mutation in the interval between *LEU1* and *TRP5* was coconverted in about 40% of the Leu^+ Trp^+ recombinants (GOLIN and FALCO 1988). The authors suggest that coincident conversion may involve extensive heteroduplex DNA; a long region of gap repair is also consistent with their results. The majority (about 80%) of Leu^+ Trp^+ recombinants displaying coconversion of the intervening marker were not homozygous for markers distal to *TRP5* and thus could not have resulted from a break-and-replicate pathway of recombination. It should be noted, however, that it would be impossible to generate recombinants of the type selected by a break-and-replicate pathway since the production of a Leu^+ Trp^+ recombinant requires conversion of one (but not both) alleles at both *LEU1* and *TRP5*. GOLIN and TAMPE (1988) have reported that coincident conversion also occurs at unlinked loci; for example, coincident conversions at *LEU1* (on chromosome *VII*) and *TYR1* (on chromosome *II*) were 4–8 times more frequent than expected for independent events. Coincident conversions of unlinked markers indicate the existence of a subpopulation of cells that undergo recombination at a high frequency.

Are *HOT1*-promoted events different from spontaneous events? In strains heterozygous for *HOT1*, *HOT1* defines the chromosome on which it resides as recipient during meiotic gene conversion. The inequality of the two participating chromosomes (one with and one without *HOT1*) makes it possible to demonstrate that most Ura^- recombinants result from gene conversion and to demonstrate that the *HOT1*-containing chromosome is recipient. In strains lacking *HOT1*, recombination events presumably initiate with equal frequency on both chromosomes; consequently, recombinants homozygous for sequences on each homolog are produced with equal frequency. The

equality of the two participating chromosomes makes it impossible to determine whether the Ura$^-$ recombinants arising in control strains result from crossing over or gene conversion. It is possible (perhaps even likely) that most of the spontaneous recombinants result from the same mechanism as the Ura$^-$ recombinants arising in *HOT1*-containing strains. *HOT1* may act only to increase the frequency of the events and to effect some alteration in their distribution.

His$^+$ recombinants may also result from a similar mechanism in both control and *HOT1*-containing strains. In both cases, the vast majority of His$^+$ recombinants result from gene conversion (as opposed to crossing over) (VOELKEL-MEIMAN and ROEDER 1990); in control strains, it is impossible to determine whether the initiating chromosome is donor or recipient. There are, however, some differences between the His$^+$ recombinants arising in control strains and those derived from *HOT1*-containing strains. In control strains, the frequency of His prototrophs resulting from simple conversion is slightly increased and the frequencies of conversions associated with crossing over and of coincident conversions at *HIS4* and *URA3* are decreased (about 2-fold). These minor differences do not preclude the possibility that spontaneous and *HOT1*-promoted events share a common mode of initiation (*e.g.*, a double-strand break).

Summary: The results presented here demonstrate that *HOT1* promotes conversion events in which all markers distal to the site of *HOT1* insertion are converted. These results can most simply be explained by a break-and-replicate pathway of recombination.

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