DNA Synthesis Errors Associated With Double-Strand-Break Repair

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ABSTRACT

Repair of a site-specific double-strand DNA break (DSB) resulted in increased reversion frequency for a nearby allele. Site-specific DSBs were introduced into the genome of *Saccharomyces cerevisiae* by the endonuclease encoded by the *HO* gene. Expression of the *HO* gene from a galactose-inducible promoter allowed efficient DNA cleavage at a single site in large populations of cells. To determine whether the DNA synthesis associated with repair of DSBs has a higher error rate than that associated with genome duplication, HO-induced DSBs were generated 0.3 kb from revertible alleles of *trp1*. The reversion rate of the *trp1* alleles was ~100-fold higher among cells that had experienced an *HO* cut than among uninduced cells. The reverted allele was found predominantly on the chromosome that experienced the DNA cleavage.

DNA synthesis is required not only to duplicate the genome but also for the processes of DNA damage repair and genetic recombination. Cells use a variety of DNA polymerases to perform different tasks, and defining the role of each of the polymerases in these processes is a focus of current research (BUDD *et al.* 1989; WANG *et al.* 1993; BUDD and CAMPBELL 1995). The high fidelity with which genomes are replicated reflects both the low frequency of errors made by DNA polymerases and the presence of mechanisms that detect and repair some classes of error (FRIEDBERG 1988; BECKMAN and LOEB 1993). The spontaneous mutation rate includes the unrepaired errors made during replication, DNA damage repair, and recombination. The relative contributions of these processes has not been established. We present here experiments that measure the fidelity of the DNA synthesis associated with recombination.

Replication errors are commonly detected as the production of novel mutants or the reversion of defective alleles. These assays detect the sum of all pathways that can yield DNA alterations and hence probably overestimate the true rate of unrepaired misincorporation during DNA replication. DNA synthesis associated with repair of damaged bases can be divided into two categories. Processes that lead to replication across damaged bases (*trans*-lesion synthesis) are designated error-prone repair. In contrast, processes in which the lesion is removed and replaced by copying an undamaged strand are designated error-free repair. Recombinational repair of DNA damage, because it involves replacing the damaged DNA with a copy of a homologous sequence, is one of the error-free pathways.

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In the experiments reported here, we examined whether the DNA synthesis associated with double-strand-break (DSB) repair has a higher error rate than the basal rate associated with genome duplication. To avoid the nonspecific DNA damage that would result from generating DSBs by chemical or radiation treatment, we used the endonuclease encoded by the *HO* gene of *Saccharomyces cerevisiae* to make a single site-specific cut. The normal role of this enzyme is to initiate the site-specific gene conversion process that changes the allele at the locus that controls mating type in this yeast, *MAT* (reviewed by HERSKOWITZ *et al.* 1992). The HO endonuclease cleaves the *MAT* locus and begins a process that results in replacing the resident *MAT* allele (*MAT***a** or *MAT*α) with a copy of silent *MAT* sequences present at loci designated *HML* and *HMR*. The HO recognition site has been defined (NICKOLOFF *et al.* 1986) and the *HO* gene placed under the control of a galactose-regulatable promoter (JENSEN and HERSKOWITZ 1984). Together, these tools allow the efficient introduction of a site-specific DNA cleavage in the yeast genome. Experiments monitoring the consequences of HO-induced cleavages on a variety of meiotic and mitotic recombination systems have been published (KOLODKIN *et al.* 1986; NICKOLOFF *et al.* 1986; RUDIN and HABER 1988; NICKOLOFF *et al.* 1989; RAY *et al.* 1989; MCGILL *et al.* 1993).

We created a genetic interval combining the *trp1* and *his3* genes that allows detailed genetic analysis of mitotic recombination events between homologous chromosomes. We previously published an analysis of spontaneous recombination in this interval (MCGILL *et al.* 1990). We also applied this system to the analysis of recombination events initiated by a protein that cleaves one strand of DNA at a site inserted in this interval (STRATHERN *et al.* 1991) and the analysis of mitotic interchromosomal

TABLE 1

Yeast strains

Strain	Genotype	Source
Haploids		
GRY1079	*MAT***a**-*inc*::[*trp1-488* HO-site *his3-192*] *cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52*	McGill *et al.* (1993)
GRY1279	*MAT***a**-*inc*::[*trp1-fsA* HO-site *his3-192*] *cry1 leu2-Δ1 ade2-101 lsy2-801 trp1-Δ1 his3-Δ200 ura3-52*	This study
GRY1281	*MAT***a**-*inc*::[*trp1-fsT* HO-site *his3-192*] *cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52*	This study
GRY1197	*MATα-inc*::[*trp1-488 his3-621*] *leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	This study
GRY1282	*MATα-inc*::[*trp1-fsA his3-621*] *leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	This study
GRY1283	*MATα-inc*:: [*trp1-fsT his3-621*] *leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	This study
GRY770	*MAT***a** *arg4-17 lys5 trp1-Δ1*	This study
GRY772	*MATα arg4-17 lys5 trp1-Δ1*	This study
DG7023617C	*MAT***a** *his3-532*	Obtained from G. Fink
DG7033618C	*MATα his3-532*	Obtained from G. Fink
DC14	*MAT***a** *his1*	Cold Spring Harbor Laboratory
DC17	*MATα his1*	Cold Spring Harbor Laboratory
Diploids		
GRY1198	GRY1079 × GRY1197 [pGALHO]	This study
GRY1199	GRY1079 × GRY1197 [pGAL1acZ]	This study
GRY1292	GRY1279 × GRY1282 [pGALHO]	This study
GRY1293	GRY1279 × GRY1282 [pGAL1acZ]	This study
GRY1296	GRY1281 × GRY1283 [pGALHO]	This study
GRY1297	GRY1281 × GRY1283 [pGAL1acZ]	This study

recombination initiated by DSBs generated by HO endonuclease cleavage at a synthetic site (McGill *et al.* 1993). In this report, we monitored the effect of DSB repair on the reversion rate of mutant *trp1* alleles adjacent to the site of cleavage and found that recombinational repair is mutagenic.

MATERIALS AND METHODS

Yeast strains: The yeast strains used are listed in Table 1. The position of the alleles is given in Figure 1 and described in McGill *et al.* (1990). The basic *TRP1-HIS3* module is shown in Figure 1. The HO recognition site is a 30-base oligonucleotide inserted into the polylinker region between *trp1* and *his3* (McGill *et al.* 1993). HO endonuclease was provided from the galactose-inducible plasmid pGALHO (Jensen and Herskowitz 1984). The *trp1* frameshift alleles were made by cloning a 20-bp oligonucleotide [a duplex between 5′AGCTCGGCCATTTTTTGGCC (oligo #8664) and 5′AGCTGGCCAAAAAATGGCCG (oligo #8668)] into the *Hin*dIII site in the *TRP1* gene. The two orientations of the oligonucleotide were designated *trp1-fsA* and *trp1-fsT* (see text).

Genetic analysis: Coupling of the *TRP1* revertant alleles to *MAT* and *CRY1* was established by classical genetics as described in McGill *et al.* (1990). Before and after growth on galactose, several hundred Ura$^+$ colonies were tested for the presence of the tester alleles. Specifically, the diploids were tested for mating phenotype to determine whether they retained the **a**/α mating phenotype indicative of heterozygosity of *MAT***a** and *MATα*, and they were tested for heterozygosity of the *his3* alleles by scoring their ability to give rise to His$^+$ recombinants. Those diploids that were heterozygous for the *his3* alleles and carried the pGALHO plasmid were tested for the presence of the HO cleavage site by monitoring the ability of growth on galactose to promote the formation of His$^+$ recombinants.

Media: The media in these experiments were prepared as described in Sherman *et al.* (1986) and McGill *et al.* (1990). Galactose induction was performed by shifting cells from synthetic complete medium minus uracil plus 5% raffinose (SC − Ura + raffinose) to SC − Ura plus 2% galactose overnight. Aliquots were titered and plated to detect His$^+$ recombinants and Trp$^+$ revertants. Frequencies were determined from the median value of 11 independent cultures. The sequenced revertants were isolated from independent liquid cultures or from patches replica-plated to SC − Ura + galactose overnight and then replica-plated to SC-Trp. A single Trp$^+$ revertant was picked from each patch.

Sequencing: The sequence of the revertants was determined by directly sequencing the PCR product from haploid segregants carrying the revertant allele as described by Sutton and Liebman (1992). Yeast DNA was prepared by a modification of methods described by Davis *et al.* (1986) as detailed in Strathern and Higgins (1991). One PCR primer (#6977) included *TRP1* sequences (5′CCCCGGCCGAA**TTCAGTGCAGCTTCACAGAAACCTC**; *TRP1* homology in bold), whereas the second primer (#6976) was complementary to the promoter region of *HIS3* (5′CCCCAGCTGCTCG**AGAGTCATCCGCTAGGTGG**; *HIS3* homology in bold). The PCR products were separated on an agarose gel (1.5% NuSieve, 1.0% SeaPlaque FMC Bioproducts), and the gel slice containing the double-stranded produce was melted and diluted fivefold into H_2O; 1 μl of this solution was used in a 100-μl asymmetric PCR (1:100 ratio of the two oligonucleotides). After two rounds of chromatography through spin dialysis columns to eliminate the primers, the PCR products were then used as templates for sequencing. Sequenced revertants come from independent cultures.

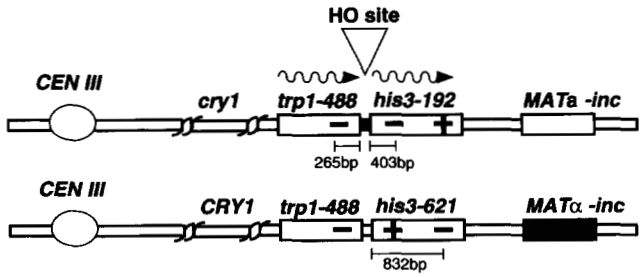

FIGURE 1.—Physical description of the *TRP1-HIS3* interval. The *TRP1* and *HIS3* genes were inserted into an *Eco*RI site centromere proximal to *MAT*. The arrows indicate the direction of transcription of the genes. Mutations in the *HIS3* gene were made by filling in the *Nde*I site at codon 64 (*his3–192*) or by filling in the *Asp*I site at codon 207 (*his3–621*). Both *MAT* alleles (*MATα-inc* and *MATa-inc*) are resistant to cutting by HO endonuclease. The HO site was inserted into a polylinker between *trp1* and *his3* on the *cry1 MATa-inc* chromosome. Both chromosomes carry the same *trp1* mutation (*trp1–488*, *trp1-fsA*, or *trp1-fsT*). The *trp1–488* allele was made by site-directed oligonucleotide mutagenesis to change two bases and create an *Spe*I site (from ACTGGG to AC*TAGT*) with an in-frame stop codon at codon 163 (*trp1–488*). The *trp1-fsA* and *trp1-fsT* frameshift alleles were made by insertion of a 20-base oligonucleotide at the *Hin*dIII site.

RESULTS

The design of these experiments involves using HO endonuclease to create a unique DSB on one chromosome in a diploid and monitoring the fidelity of the repair synthesis associated with repair of that break. In yeast, DSB repair is almost exclusively accomplished by recombination in which a homologous sequence is used as a template for DNA synthesis spanning the site of the break. In our experiments, the HO site was placed within a region specifically constructed for recombination studies that includes the *trp1* and *his3* genes inserted on chromosome *III* near *MAT* (McGILL *et al.* 1990). In each experiment, the same *trp1* allele (*trp1–488*, *trp1-fsA*, or *trp1-fsT*) was present on both chromosomes in these diploids (Figure 1). The HO site was present on only one chromosome between the *trp1* and *his3* genes; therefore, recombinational repair of the DSB caused by HO cleavage resulted in the loss of the HO site. Each cell could undergo only one HO-induced recombination event. Reversion of a *trp1* allele adjacent to the HO site was monitored under HO-induced and -repressed conditions. The normal sites for HO recognition in the *MAT* alleles had been mutated so that they would not be substrates (*MATa-inc* and *MATα-inc*).

The presence of two different alleles of *his3* provided a means to monitor HO endonuclease-induced recombination. We previously demonstrated the efficient production of *HIS3* recombinants in strains carrying this configuration of the HO site and the *his3* alleles. Up to 3% of the cells in the culture were His^+ after 12 hr of induction of the pGALHO plasmid. The His^+ cells represented ~10% of the cells that had undergone an HO-induced recombination event (McGILL *et al.* 1993).

TRP1 reversion was elevated among the HO-induced recombinants: The yeast strain shown in Figure 1 was transformed with pGALHO, a plasmid carrying *HO* under the control of a galactose-regulatable promoter (JENSEN and HERSKOWITZ 1984). The control strain carried a related plasmid (pGALlacZ) that expressed β-galactosidase under the control of the *GAL1* promoter. Reversion of the *trp1–488* allele was measured for cultures grown in the noninducing carbon source (raffinose) and again 12–16 hr after shifting the cultures to galactose-containing medium. The results (Table 2) indicate a 33-fold increase in the frequency of Trp^+ cells for the pGALHO strain. At this time point, 10% of the cells had experienced an HO-induced recombination as determined by loss of the HO site or a change in the configuration of other alleles in the *cry1 trp1-his3 MAT* interval. Combined, these numbers suggest over 300-fold higher reversion frequency among the cells that had undergone this DSB repair. The presence of the HO site could be monitored by a *Sma*I site (McGILL *et al.* 1993). At a time when only 10% of the total cells had lost the HO site, 90% of the *trp1–488* revertants lost the HO site (data not shown). This indicates that repair of the DSB has a direct role in the production of the revertants. Growth on galactose did not increase the frequency of Trp^+ cells in the pGALlacZ control strain.

The reverted allele was on the cut chromosome: Various models for DSB repair (discussed below) have postulated that the newly synthesized DNA and, hence, any errors made during that synthesis, could be inherited by the cut chromosome, the uncut chromosome, or both chromosomes. In cases where the outside markers (*cry1* and *MAT*) retained the coupling in the parental strain (Figure 1), it was possible to define the chromosome that had the HO cleavage and the chromosome that was uncut. The configuration of the *cry1* and *MAT* alleles and the chromosome carrying the reverted allele was determined for spontaneous and HO-induced revertants. Each revertant came from an independent culture. For the spontaneous revertants, the results were as expected; most (35/38) of the revertants retained the parental configuration of the outside markers, and the revertant allele was found equally often on the two chromosomes (Table 3). The results for the HO-induced reversion events showed a strikingly nonrandom distribution. As expected, the HO-induced revertants were associated with a high proportion of crossing over associated with the repair of the DSB. In these cases, the chromosome on which the reversion occurred could not be determined, because crossing over could have occurred to the left or right of the revertant allele. In the 23 cases where crossing over did not occur, all of the revertant alleles were recovered on the chromosome that had been cleaved by HO and repaired. Possible origins for this nonrandom distribution of the revertants are discussed below.

Several different revertant alleles were recovered: The *trp1–488* allele is the result of two base changes that make a nonsense (amber) mutation and

TABLE 2

Reversion frequency

	Before induction (raffinose)	After induction (galactose)
Reversion of *trp1-488*		
GRY1198 (pGALHO)		(10% HO induction)
Trp^+	0.3×10^{-8}	11×10^{-8}
His^+	0.4×10^{-4}	68×10^{-4}
GRY1199 (pGAL1acZ)		
Trp^+	0.9×10^{-8}	1.1×10^{-8}
His^+	5.5×10^{-4}	4.3×10^{-4}
Reversion of *trp1-fsA*		
GRY1292 (pGALHO)		(8% HO induction)
Trp^+	0.3×10^{-8}	8.6×10^{-8}
His^+	0.2×10^{-4}	68×10^{-4}
GRY1293 (pGAL1acZ)		
Trp^+	0.4×10^{-8}	0.2×10^{-8}
His^+	1.1×10^{-4}	1.4×10^{-4}
Reversion of *trp1-fsT*		
GRY1296 (pGALHO)		(8% HO induction)
Trp^+	0.1×10^{-8}	20.5×10^{-8}
His^+	0.2×10^{-4}	77×10^{-4}
GRY1297 (pGAL1acZ)		
Trp^+	0.1×10^{-8}	0.1×10^{-8}
His^+	0.1×10^{-4}	0.1×10^{-4}

Frequencies represent median value for 11 independent cultures.

generate a recognition site for the *Spe*I endonuclease. Although unlinked nonsense suppressors can be selected that will suppress this mutation in haploids, we observed that suppression was poor in diploids (data not shown). No unlinked suppressor was obtained under the conditions of this experiment among the induced or spontaneous revertants. Confirmation that the reversion occurred at the site of the nonsense mutation was obtained by restriction analysis (loss of the *Spe*I site) of DNA derived from haploid segregants carrying the novel allele from 11 spontaneous and 49 induced revertants. Twenty independent HO-induced revertants were sequenced (Figure 2) and included five different sequence alterations encoding four different amino acids. Nineteen of the sequenced revertants were the result of transversions.

TABLE 3

Distribution of the revertant allele between the two chromosomes

	Chromosome with the revertant allele	
	CRY1 MATα-inc	*cry1 MAT***a***-inc*
trp1-488 revertants		
Spontaneous	19	15
HO induced	0	23
trp1-fsA revertants		
Spontaneous	8	10
HO induced	4	26
trp1-fsT revertants		
Spontaneous	6	8
HO induced	1	45

Reversion of frameshift mutations: The nature of the polymerase error necessary to generate a frameshift could be different than the error that generates a base-substitution mutation. Therefore, we performed a similar analysis of the reversion of frameshift mutations in the vicinity of DSB-repair events. Two *trp1* frameshift alleles were made by inserting a 20-base oligonucleotide into the *Hin*dIII site in *TRP1* (Figure 3). In one orientation, the oligonucleotide introduced six adenines in the coding strand, and the resulting frameshift allele is called *trp1-fsA*. The frameshift allele with the oligonucleotide in the other orientation has six thymidines in the coding strand and is designated *trp1-fsT*. This region of the Trp1 protein is very tolerant of alterations. We have identified over 30 different kinds of revertant alleles of mutants in this interval.

Reversion of the *trp1-fsA* and *trp1-fsT* alleles was stimulated by the induction of a nearby DSB (Table 2). Shifting the pGALHO strain (GRY1292) from raffinose to galactose medium resulted in a 30-fold increase in the frequency of Trp^+ revertants in a population that included ~8% cells that had undergone DSB repair. Combined, these results suggest a 360-fold increase in the reversion frequency of the *trp1-fsA* mutation among cells that had undergone DSB repair. In contrast, the pGALlacZ control strain (GRY1293) showed no increase in Trp^+ revertants upon growth in galactose. Similarly, the strain containing the *trp1-fsT* allele and pGALHO showed a several hundred fold increase in

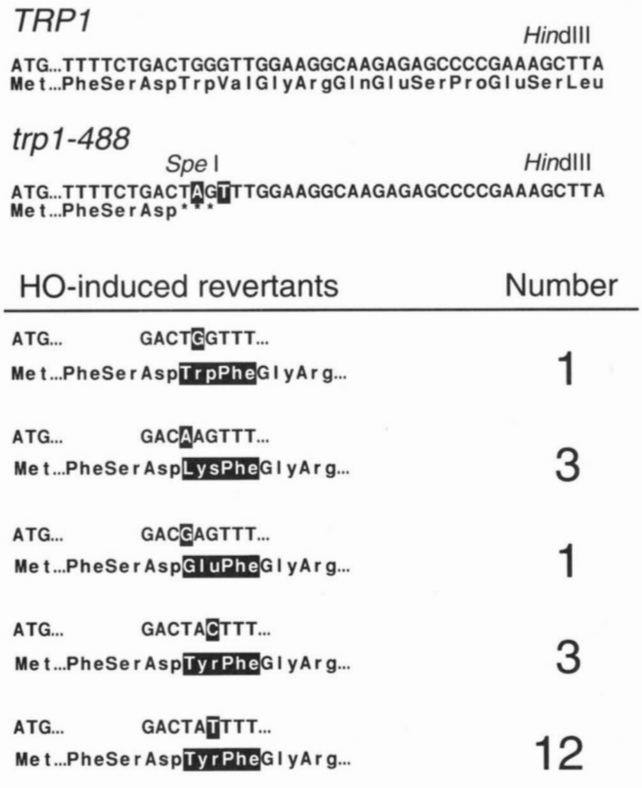

FIGURE 2.—Revertants of the *trp1–488* nonsense allele. The sequences of the *TRP1* and *trp1–488* alleles are shown along with the sequences of 20 independent Trp$^+$ revertants from cultures in which HO endonuclease was expressed.

the frequency of Trp$^+$ revertants among the cells that had undergone DSB repair (Table 2).

The sequence of the revertant alleles recovered from the frameshift mutants revealed a variety of reversion mechanisms (Figure 3). We obtained revertants of the *trp1-fsA* and *trp1-fsT* alleles that resulted from the addition of a base to the array of six adenines or thymidines, respectively. This class of reversion was readily detected because a single-base insertion at this position destroyed a *Pfl*MI site and generated a *Bst*XI site. We anticipated that this region might present a favorable place for polymerase slippage; however, support for the simple view that this class of revertants would be as common when the As were on the coding strand as when they were on the noncoding strand was not found. Single-base insertions in this interval represented the majority event (12/14) among HO-induced revertants and spontaneous events (6/9) for the *trp1-fsA* mutation. For the oligonucleotide in the other orientation (six thymidines in the coding strand: *trp1-fsT*), only 3 of 27 were the result of insertions of an extra base to this array of Ts. The majority (17/22 sequenced) of the HO-induced revertants of the *trp1-fsT* allele was the result of single-base insertions at another specific position. This insertion turns a near palindrome into a perfect palindrome, suggesting a possible mechanism for the origin of these revertants. RIPLEY (1982) has proposed that quasipalindromic DNA sequences can provide sites where the growing DNA chain can fold back and copy itself briefly

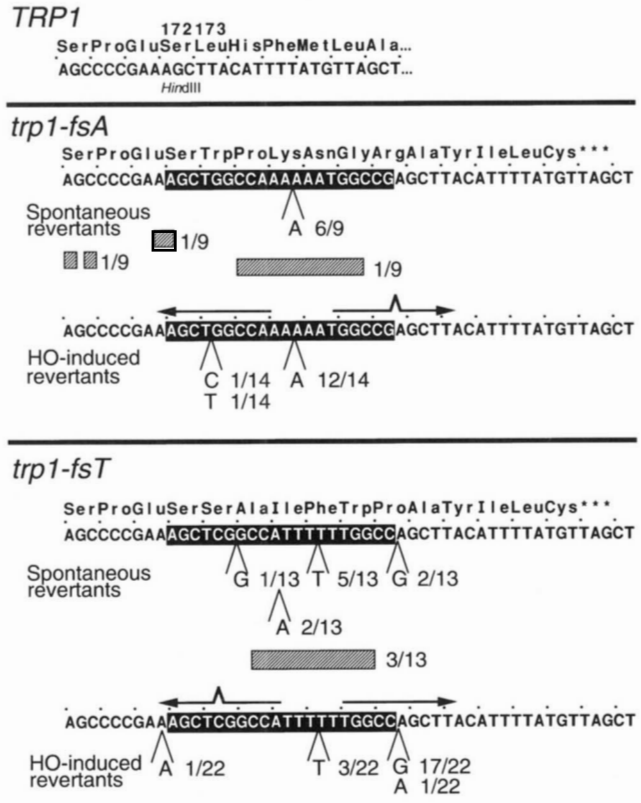

FIGURE 3.—Revertants of the *trp1-fsA* and *trp1-fsT* frameshift alleles. The sequences of the *TRP1*, *trp1-fsA*, and *trp1-fsT* alleles are shown along with the sequences of independent Trp$^+$ spontaneous and HO-induced revertants. The position of single-base insertions is indicated along with the number of such events among the sequenced revertants. Deletions are noted as cross-hatched bars. Arrows indicate a quasipalindrome.

before continuing to extend in the proper direction, thus generating frameshift mutations or, in the case of *trp1-fsT*, a revertant. This class of revertant comprised only 2 of 13 of the spontaneous revertants of *trp1-fsT*. The insertion to make the corresponding palindrome was found in only 1 of 14 HO-induced revertants of *trp1-fsA* and was not found among the spontaneous revertants of that allele. The different spectra of HO-induced revertants of *trp1-fsA* and *trp1-fsT* suggest that the two strands of DNA at that position are treated differently in the DSB repair process.

DISCUSSION

DNA synthesis is a remarkably high fidelity process. Most DNA synthesis is associated with duplication of the genome, and the fidelity of that process is augmented by mismatch correction mechanisms that edit out insertion errors. DNA synthesis associated with DNA damage repair is usually classified as error prone if it involves synthesis across the DNA lesion or error free if it involves removal of the lesion and copying of an undamaged template. Recombinational repair is considered to be a subset of the error-free repair processes. The experiments described here monitored the rever-

sion of a mutation located over 300 bases from the site of an inducible DSB. We assume that cleavage by HO caused no direct damage at the site of the *trp1–488*, *trp1-fsA*, or *trp1-fsT* alleles and that the observed elevated frequency of revertants among the HO-induced recombinants was the result of errors made during the DNA synthesis associated with the DSB-repair process.

We observed over a 300-fold increase in the frequency of Trp^+ revertants among cells that had undergone HO-induced DSB repair. This frequency can be used to make a rough estimate of the error rate. The number of cells increased only about twofold during the induction. Further, each cell could undergo only one round of HO cutting and DSB repair because the HO recognition site was lost during the repair process. Thus, the observed frequency, $\sim 10^{-6}$ among cells that had a recombinational repair event, is an approximate overall rate per cell. To determine the rate per base pair synthesized, it is necessary to know what fraction of the time the recombinational DNA synthesis spanned the mutation and what fraction of errors would result in a reversion. It is not certain what fraction of the repair events included DNA synthesis extending 0.3 kb from the HO site to the *trp1* alleles. HO cleavage at the same position in a strain heterozygous for the *trp1–488* allele resulted in over 30% gene conversion of that allele (McGill *et al.* 1993), consistent with a high proportion of events requiring synthesis of at least one strand beyond that distance. At least five of the possible nine single-base alterations of the *trp1–488* nonsense allele revert the Trp^- phenotype. Combined, these factors suggest that the unrepaired misincorporation rate for the DNA polymerase(s) involved in DSB repair is between 10^{-5} and 10^{-6} per base synthesized.

Five different revertant alleles of *trp1–488* were obtained from the pool of DSB-induced cells. All the revertants were the result of base changes in the nonsense codon in *trp1–488*. Although the complete spectrum of base changes in this codon that allow function of the Trp1 protein is not known, it is interesting that the majority (19/20) of revertants were transversions. Additional experiments will be required to determine whether the base-substitution errors associated with DSB repair have a different spectrum than spontaneous events.

In contrast to the spontaneous revertants that were found equally on both chromosomes, it is remarkable that nearly all the revertants were on the chromosome that had the DSB, indicating that the elevated revertant frequency was not the result of a general increase in mutation rate. We entertain two models for how the revertants could be constrained to appear on only the chromosome that was originally cut by HO. One view is that the DSB-repair intermediates that are not associated with crossing over are resolved by a topoisomerase rather than by cleavage of Holliday junctions (Nasmyth 1982; Hastings 1988; Thaler and Stahl 1988; McGill *et al.* 1989). In this view, all of the newly synthesized

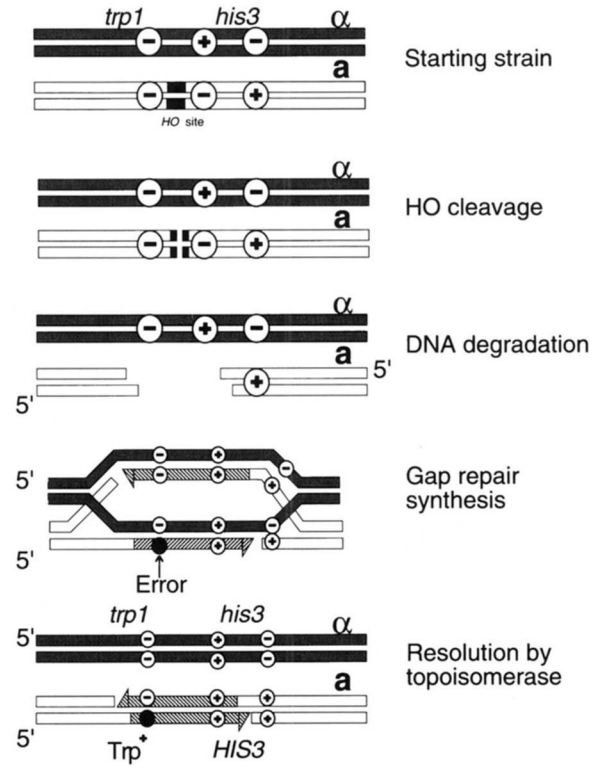

Figure 4.—DSB-initiated recombination. The DNA strands of the broken chromosome are white, whereas the uncut chromosome used as a template for repair is black. Newly synthesized DNA, copied from the uncut chromosome, is cross-hatched. The lengths of the double-strand gap, the half-gaps, and the regions of symmetric strand exchange can be variable. Resolution without crossing over by a topoisomerase transfers all of the newly synthesized DNA (and, hence, all the errors) to the cut chromosome.

DNA (and, hence, all the replication errors) associated with the repair are inherited by the cut chromosome (Figure 4). This would be true whether the error was made by the polymerase replicating left to right across *trp1* (to replace degradation of the strand ending 3′ at the HO site as shown in Figure 4) or the error was made by the polymerase replicating right to left through *trp1* (to replace degradation of the strand ending 5′ at the HO site as shown in Figure 5).

A second view for how all the errors could be inherited by the chromosome that was cut is analogous to the mechanism of directed mismatch repair. Mismatches that would be inherited by the uncut chromosome could be preferentially repaired in the direction that restores the mutant *trp1* allele, whereas mismatches that would be inherited by the cut chromosome either are not detected or are repaired without strand bias. This difference could be accomplished by exploiting a difference between the two chromosomes. Resolution of the DSB-repair intermediate by cleavage of the strand junctions leaves one strand of the uncut chromosome intact (Figure 5), whereas the newly synthesized DNA (and, hence, any errors) is on a strand that has a strand discontinuity or nick. The presence of the nick may give the mismatch-correction system a strand bias fa-

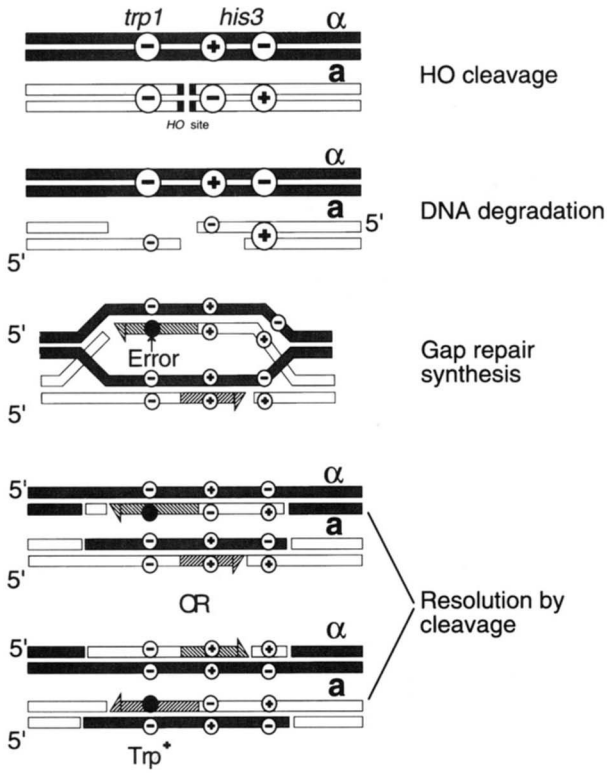

FIGURE 5.—Resolution without crossing over by cleavage of Holliday junctions results in both the cut chromosome and the repair template chromosome receiving newly synthesized DNA (and, hence, the potential for errors). Nicks in the chromosomal DNA may confer a strand bias in the repair of mismatches created by polymerase errors.

voring the sequence on the uncut strand, as is seen in the *Escherichia coli mutS* system (LÄNGLE-ROUAULT *et al.* 1987; LAHUE *et al.* 1989; MODRICH 1991). In contrast, cleavage of the strand junctions could leave nicks in both the strands of the chromosome that originally had the HO site. Under these circumstances, mismatch repair may not have directionality and thus could not contribute to the fidelity of repair synthesis on the recipient chromosome. We will test this model by determining whether defects in mismatch correction result in the production of revertants inherited on the uncut chromosome. This same mechanism may contribute to the paucity of aberrant 5:3 meiotic tetrads, a class of recombinants showing retention of a heteroduplex on the donor chromosome as defined by no crossing over of outside markers (FOGEL *et al.* 1981).

The different spectra of HO-induced revertants from the *trp1-fsA* and *trp1-fsT* alleles suggest that the two strands of DNA at that position are treated differently in the DSB-repair process. The simple copying of one strand through the *trp1-fsA* allele should be the same as copying the other strand through the *trp1-fsT* allele. Two classes of revertants account for the majority of the DSB-induced revertants: the addition of a base to the repeat of six bases or the addition of a base to form an extended palindrome. If the two strands were treated equally, one might expect the ratio of these two types of revertants to be the same for the *trp1-fsT* and *trp1-fsA* alleles. The observed asymmetry is consistent with the proposal that most of the repair synthesis that spans the mutation site is confined to a specific strand. The extent of DNA synthesis associated with DSB repair reflects in part the extent of DNA degradation that occurred at the site of the break. There appears to be more extensive degradation of the strand that ends 5′ at a DSB than of the 3′-ended strand (WHITE and HABER 1990). This effect would require a similar asymmetry in the repair synthesis. In this view, the majority of the DNA synthesis across *trp1* to repair a DSB on the right side of *trp1* would result from synthesis right to left across *trp1* (Figure 5). However, this view is not consistent with the suggestion that the most common reversion of the *trp1-fsT* allele (adding a base to make a palindrome) is the result of copying back on itself. For the *trp1-fsT* allele, adding a base by this mechanism would occur when synthesis is from left to right. The abundance of *trp1-fsT* revertants of the palindrome class indicates that, at least some of the time, degradation of the 3′ strand extends >0.3 kb from the DSB. Our data do not distinguish whether the difference between the reversion spectra of *trp1-fsT* and *trp1-fsA* is the result of more extensive 3′ than 5′ degradation and resynthesis from alternative interpretations. For example, the majority of DNA synthesis could be right to left across *trp1* (replacing 5′ degradation) and the poly A template might be more "slippery" than the poly T template.

The experiments presented here demonstrate that DNA synthesis associated with recombinational repair of DSBs has an elevated error rate relative to the net spontaneous rate. The spontaneous mutation (or reversion) rate reflects the sum of all the DNA synthesis and correction processes occurring in the cell. It is unclear whether the observed rate is dominated by unrepaired errors made in the replication fork or by errors made during repair of DNA damage. The replication fork involves at least three different DNA polymerases and a variety of associated factors that promote the fidelity of replication (KORNBERG and BAKER 1991). DNA synthesis associated with damage repair can be divided into two categories. Synthesis across the damaged template is error prone because mispairing can occur with the damaged base or abasic site. Alternatively, when the damaged DNA has been removed, resynthesis uses an undamaged strand. Recombinational repair is a subset of the error-free pathway. The relative contributions of errors made by these different classes of DNA synthesis to mutation rates has not been determined. The approach taken here allows us to focus on the DNA synthesis associated with recombinational repair. The assay that we have described may allow the identification of the polymerases and fidelity factors that participate in this process by identifying mutations that reduce or elevate the DNA synthesis error rate in the vicinity of a DSB.

It has been known for a long time that DNA damage

is recombinagenic. These data suggest that recombination may be mutagenic. MAGNI and VON BORSTEL (1962) demonstrated that meiosis is mutagenic and suggested that the elevated mutation rate is associated with recombination. MAGNI (1964) and ESPOSITO and BRUSCHI (1993) demonstrated that diploids give rise to homozygous mutations at rates that exceed the mutation rate times the rate of homozygosis of existing mutations. They concluded that mutagenesis and recombination are coupled. These previous experiments demonstrating a correlation of mutagenesis and crossing over did not establish the causal relationship, that is, which came first, the mutation or the recombination event? In the experiments presented here, the DSB used to induce recombination is 0.3 kb from the marker that is monitored for reversion, indicating that it is the recombinational repair that is mutagenic. This assay opens the door for an investigation of the factors involved in the fidelity of DNA synthesis associated with recombinational repair mechanisms.

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