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Induction of Recombination between Homologous and Diverged DNAs by Double-Strand Gaps and Breaks and Role of Mismatch Repair

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Sequence homology is expected to influence recombination. To further understand mechanisms of recombination and the impact of reduced homology, we examined recombination during transformation between plasmid-borne DNA flanking a double-strand break (DSB) or gap and its chromosomal homolog. Previous reports have concentrated on spontaneous recombination or initiation by undefined lesions. Sequence divergence of approximately 16% reduced transformation frequencies by at least 10-fold. Gene conversion patterns associated with double-strand gap repair of episomal plasmids or with plasmid integration were analyzed by restriction endonuclease mapping and DNA sequencing. For episomal plasmids carrying homeologous DNA, at least one input end was always preserved beyond 10 bp, whereas for plasmids carrying homologous DNA, both input ends were converted beyond 80 bp in 60% of the transformants. The system allowed the recovery of transformants carrying mixtures of recombinant molecules that might arise if heteroduplex DNA—a presumed recombination intermediate—escapes mismatch repair. Gene conversion involving homologous DNAs frequently involved DNA mismatch repair, directed to a broken strand. A mutation in the *PMS1* mismatch repair gene significantly increased the fraction of transformants carrying a mixture of plasmids for homologous DNAs, indicating that *PMS1* can participate in DSB-initiated recombination. Since nearly all transformants involving homeologous DNAs carried a single recombinant plasmid in both Pms$^+$ and Pms$^-$ strains, stable heteroduplex DNA appears less likely than for homologous DNAs. Regardless of homology, gene conversion does not appear to occur by nucleolytic expansion of a DSB to a gap prior to recombination. The results with homeologous DNAs are consistent with a recombinational repair model that we propose does not require the formation of stable heteroduplex DNA but instead involves other homology-dependent interactions that allow recombination-dependent DNA synthesis.

Double-strand breaks (DSBs) in DNA are potent inducers of homologous recombination. DSBs can result from DNA damage or as part of cell development, such as meiosis (50, 57) or mating-type switching (49) in yeast cells. The elaboration of recombination mechanisms has been considerably enhanced through studies of DSB repair (53).

The DSB ends are expected to play a prominent role in their repair in terms of genetic outcome and opportunities for repair. For example, models for DSB-induced recombination invoke the interaction of DNA from either one end (39) or both ends (39, 52) generated by the DSB with a homologous, intact DNA duplex (summarized in reference 53). The former leads only to nonreciprocal recombination, whereas the latter can also lead to crossing over. Absence of homology at the ends affects both the mechanism of recombination (11) and the cellular response to a break (4). Through the use of molecules with reduced homology at the ends and mutants altered in mismatch repair, we anticipated the opportunity to characterize the role of ends in DSB-induced recombination.

Insight into recombination (2, 3, 15, 23) has been gained through the use of DNA sequences (DNAs) that are diverged, i.e., homeologous. This type of recombination has potential as a tool for protein engineering (35). Such recombination appears to be an important source of some genetic diseases (17, 26, 48) and appears relevant in evolution (34, 37). Since recombinants derived from interactions between homeologous DNAs are recovered less frequently than for homologous DNAs (3, 15, 23, 41), DNA divergence could serve to inhibit recombination between related sequences such as those commonly occurring in higher organisms. The reduction in recombination frequency could result from physical constraints inherent in the recombination process itself and from homology-dependent genetic controls that restrict recombination between homeologous DNAs. For example, the rate of initiation of *recA*-mediated strand transfer in *Escherichia coli* decreases with nucleotide sequence divergence (9). DNA mismatch repair (reviewed in reference 25) can act during recombination between homeologous DNAs to further reduce the frequency. In conjugal crosses between Hfr *E. coli* and F$^-$ *Salmonella typhimurium* (approximately 20% DNA divergence), recombination in wild-type recipients is 10^5-fold lower than for crosses between *E. coli* strains. For recipients defective in DNA mismatch repair, recombination during interspecies mating is only 100-fold lower relative to intraspecies mating (37). Thus, DNA mismatch repair in bacteria controls recombinational fidelity as well as replication fidelity. In mammalian cells, the capability depends on whether the diverged DNAs are chromosomal or autonomously replicating (54).

In the yeast *Saccharomyces cerevisiae*, sequence divergence seems to be much less of a barrier to recombination than seen in bacteria. Gene conversion between homeologous DNAs (73 to 85% homology) occurs at a 10- to 20-fold-lower frequency

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than between homologous DNAs under a variety of circumstances, including meiotic recombination (29), ectopic recombination (3, 15), intraplasmid recombination between repeats (21), and recombination between mammalian P-450 genes on linearized plasmid DNA used for yeast transformation (23). In mitotic cells, there are comparable levels of reciprocal recombination associated with gene conversion for both homologous and homeologous DNAs (15). In contrast, meiotic reciprocal exchange between homeologous DNAs remains to be shown unequivocally and is at least 100-fold less frequent than between homologous DNAs (29–31, 35b). In G_1 diploid cells, DSBs induce recombination between homeologous chromosomes (41) as well as chromosome loss (40), reflecting limited recombinational repair.

Considering observations with bacteria, DNA mismatch repair might be expected to limit recombination between homeologous DNAs in *S. cerevisiae*. However, this does not appear to be the case for the *PMS1* gene (56), which is homologous to the *mutL* and *hexB* mismatch repair genes of bacteria (20). In a *pms1* mutant background, heteroallelic recombination increased three- to fivefold compared with the wild type, but the basis for the increase was not apparent. Mitotic, ectopic recombination between *SAM1* and *SAM2* genes and between *SAM1* genes was elevated by the same factor (3). Although radiation-induced heteroallelic recombination between homeologous chromosomes was more frequent for *pms1* strains, no difference in radiation-induced aneuploidy was observed between *PMS1* and *pms1* strains (41). The importance of other recently identified mismatch repair genes in *S. cerevisiae* (27, 38) that are related to *E. coli mutS* remains to be established.

In this study, we examine DSB repair between homologous and diverged DNAs in order to understand the role that the ends can play in the genetic outcomes and the function of the *PMS1* gene in the processing of the ends. In previous studies, it was not possible to relate recombination between diverged DNAs with the initiating lesion since either they were directed at spontaneous recombination or the initiating lesion was undefined. We have examined DSB-induced recombination between transforming plasmid DNA and a chromosome. The plasmid contained a defined double-strand gap (DSG) or DSB bounded by ends that are homologous or homeologous with the target chromosomal sequence used for recombinational repair. The system allowed the recovery of mixed plasmids as might result if heteroduplex DNA was an intermediate in recombination. The consequences of diverged ends on recombination were deduced by examining changes in the ends of the plasmids accompanying a gap-filling reaction or plasmid integration. The recombinational process and the role of the *PMS1*-dependent mismatch repair system differed between diverged and homologous DNAs. The implications for the relationship between mismatch repair and recombination and models of recombination in *S. cerevisiae*, as well as the use of diverged DNAs for targeting and the development of chimeric genes, are discussed.

MATERIALS AND METHODS

Strains and plasmids. Haploid strains of *S. cerevisiae* used as transformation recipients were 1113 (*MATα his4-644 leu2 thr4 ura3-52 can1*) and isogenic *pms1*Δ (20) derivatives 1214 and 1215 (41), and 1120 (*MAT***a** *his4-S8 ura3-52 trp1*) and isogenic *pms1*Δ derivative 1220 (41). Strains 1120 and 1220 have had the normal chromosome III replaced with the divergent chromosome III of *S. carlsbergensis* (31, 41). *E. coli* DH5α was used in plasmid construction and for rescue of recombinant plasmids from yeast strains for analysis.

Plasmids used for transformation carried *URA3* and *HIS4* of *S. cerevisiae*. Plasmid pNK2004 (obtained from N. Kleckner) consists of the *HIS4* region (4.0-kb *Sac*I-*Sph*I fragment) inserted as an *Sph*I fragment into the *Sph*I site of YEp24 with the 3′ end of *HIS4* proximal to *URA3*. A derivative of pNK2004 incapable of replication in *S. cerevisiae*, YIpNK2004-Z, was constructed by deletion of the 1.8-kb *Spe*I fragment containing most of the 2μm circle DNA. YEpSDP21 was constructed by inserting the *Sph*I fragment containing *HIS4* from pNK2004 into the *Sph*I site of a derivative of YEplac195 (13) in which the *Cla*I site had been filled in with the Klenow fragment of DNA polymerase I. The fragment is oriented with the 5′ end of *HIS4* proximal to the 2μm circle DNA. YIpSDP26 was constructed by first inserting the *Sph*I fragment containing *HIS4* into YIplac211 (13) with the 5′ end of *HIS4* proximal to *URA3*. The 25-bp *Pst*I-*Sma*I fragment from the polylinker was then deleted, with the 3′ overhang having been removed with T4 DNA polymerase. YEpSDP29 was constructed from YEp SDP21 by sequentially filling in the *Cla*I (YEpSDP28) and *Esp*I sites in *HIS4* with the Klenow fragment of DNA polymerase I.

Restriction endonuclease map of *HIS4* of *S. carlsbergenesis* (*HIS4carl*). Plasmid pCB3 (obtained from M. Christman) carries a 4.6-kb *Cla*I DNA fragment from *S. carlsbergenesis* containing the *HIS4* gene homeologous to *HIS4* of *S. cerevisiae*. The fragment is inserted into the *Cla*I site of pBluescript M13+. This plasmid was used to determine a restriction map of the region (Fig. 1). Restriction endonucleases with no cleavage sites within the *HIS4* fragment are *Aat*II, *Apa*I, *Bam*HI, *Bgl*II, *Cel*II (*Esp*I), *Kpn*I, *Mlu*I, *Nar*I, *Nhe*I, *Nru*I, *Pst*I, *Sac*II, *Sph*I, *Ssp*I, *Sma*I, and *Xho*I. Cleavage sites for other restriction endonucleases were mapped relative to *Cla*I or *Eco*RI. Cleavage sites for *Msc*I and *Nde*I were each mapped relative to *Xba*I and *Eco*RI in triple digests to resolve ambiguity. Sizes of restriction fragments were measured on 1.2% agarose gels.

Yeast transformation. Yeast strains were transformed by a lithium chloride method (18) as follows. Overnight cultures grown to late log phase in YEPD medium were diluted to an optical density at 600 nm of 0.5 (approximately 10^7 cells per ml) with YEPD and supplemented with uracil and adenine to 50 μg/ml. Cells were harvested at optical density at 600 nm of 1 in a Sorvall GLC-2B clinical centrifuge (5 min at 1,800 rpm) and washed with TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA). Cell pellets were resuspended in 120 μl of 0.1 M LiCl–TE for every 2 ml of culture harvested and incubated 40 min at 30°C. Samples were prepared in 1.5-ml microcentrifuge tubes with 100 ng of transforming DNA (in ≥2 μl) and 50 μg of denatured carrier DNA (in 5 μl), as prepared by Schiestl and Gietz (43). To each tube, 120 μl of cell suspension was added, and the mixture was incubated 30 min at 30°C. Then 300 μl of a 50% polyethylene glycol 4000 solution in 0.1 M LiCl–TE was added and incubated 30 min at 30°C. The samples were then heat shocked for 15 min at 42°C. The transformed cultures were diluted twofold with water and plated on selective media. The concentration of plasmid DNA used for transformation was in the linear range of dose response. A 10-fold reduction in DNA concentration reduced transformation frequency by 10-fold (data not shown).

Restriction analysis of recombinant plasmids from yeast transformants. Plasmid-enriched DNA for transformation of *E. coli* was prepared from yeast cells as described by Ward (55). Competent *E. coli* DH5a was prepared with calcium chloride and transformed as described by Miller (24). Trans-

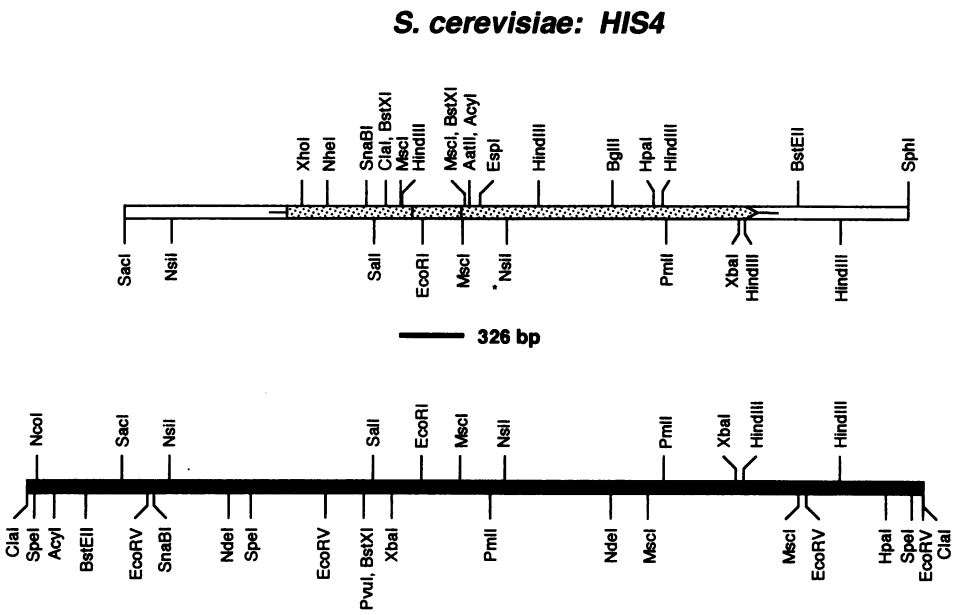

FIG. 1. Restriction endonuclease maps of *HIS4cere* and *HIS4carl*. The map for *HIS4cere* covers 4 kb and is derived from the nucleotide sequence (10). The stippled region delineates the *HIS4* open reading frame. The *Nsi*I indicated by an asterisk is not present in the published sequence. The map for *HIS4carl* was determined for this work (see Materials and Methods).

formants were selected on LB agar media with ampicillin (100 μg/ml).

Plasmid DNA was purified from *E. coli* by alkaline lysis as described by Birnboim (6) except that following the first precipitation with ethanol, the DNA pellet was dissolved in water and 10 M ammonium acetate was added to 2.5 M. The solution was placed on ice for at least 15 min and centrifuged at 14,000 rpm for 10 min at 4°C to remove the precipitate. The supernatant was removed, and the DNA was precipitated with 0.6 volume of isopropanol. The DNA was dissolved in 1 M ammonium acetate and precipitated with 2 volumes of ethanol. The DNA pellet was dissolved in TE (pH 8).

Statistical analysis. *G* tests with the Yate's correction applied (47) were used to obtain χ^2 values for 2×2 tests of independence for the effect of the Pms^- mutation on distributions of gene conversion events among transformants. Values of χ^2 for which $P \leq 0.05$ were considered significant.

DNA sequence. Sequencing of recombinant plasmid DNAs was carried out by using the PRISM DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Inc., Foster City, Calif.) on a model 373A ABI DNA sequencer according to the manufacturer's instructions. A set of four 20-mer oligonucleotide primers was used. Two primers were used for sequencing outward from the gap region in all of the plasmids examined. They were homologous to *HIS4carl* sequences internal to the gap (35a) and corresponded to the *S. cerevisiae HIS4* (*HIS4cere*) sequence from bp 1960 to 1979 and from bp 2226 to 2245 (10). The other two primers were homologous *HIS4cere* sequences corresponding to bases from 1529 to 1548 and from 2666 to 2685 (10); these were used for sequencing toward the gap for plasmids with conversion tracts that extended beyond 100 bp from the gap.

Southern analysis of yeast transformants with integrated plasmid DNA. The purpose of the Southern analysis was to determine the presence or absence of restriction sites in plasmid *HIS4* DNA integrated into the chromosome. Yeast genomic DNA, prepared as described by Hoffman and Winston (16), was digested with *Hin*dIII followed by *Sna*BI, *Spe*I, *Xho*I, or *Nhe*I, or by *Sac*I followed by *Cla*I, *Cel*II (or *Esp*I), or *Bgl*II. Digests were treated with RNase A, and approximately 60 to 100 ng of DNA was loaded per well of 0.8% agarose gels for electrophoresis. DNA was transferred to nylon membranes (Magnagraph), using a Stratagene PosiBlot pressure blotter as recommended by the manufacturer. DNA was fixed to the membrane by exposure to UV light. DNA hybridization was carried out by using a Genius kit (Boehringer Mannheim), with alkaline phosphatase detection, as recommended by the manufacturer. The digoxygenin-labeled pBR325 (150 ng for a 150-cm^2 membrane) provided in the kit was used as probe. Digestion with *Hin*dIII or *Sac*I usually yielded a 7- or 7.8-kb fragment, respectively, containing the integrated vector DNA.

RESULTS

We examined recombination initiated by a DSB or DSG during transformation of haploid strains of *S. cerevisiae* with plasmids carrying the lesion within DNA homologous or homeologous to chromosomal DNA. Two types of transformation event were examined: establishment of an episomal (2 μm) plasmid that could replicate autonomously and integration of a plasmid that could not (integrative plasmid). The fate of the ends generated by the DSB or DSG and the role of the DNA mismatch repair gene *PMS1* were of particular interest in comparing the effects of sequence divergence on recombination.

The lesions were made with restriction endonucleases within a plasmid-borne *HIS4* (or *URA3*) gene of *S. cerevisiae*. The recipient strains were wild type or mutant in *PMS1* (56) and carried either the normal chromosome III (CHRIII*cere*) or a divergent chromosome III from *S. carlsbergensis* (CHRIII*carl*)

TABLE 1. Relative transformation frequencies of plasmids cut in *HIS4cere*

Relevant genotype of recipient strain	Relative transformation frequency[a]		
	Episomal plasmid[b] cut with *Msc*I	Integrative plasmid[c] cut with:	
		*Bgl*II	*Bst*XI
CHRIII*cere*			
PMS1	0.63 (0.1)	2.8 (0.3)	4.0 (0.7)
pms1	0.65 (0.05)	1.8 (0.3)	3.4 (1.0)
CHRIII*carl*			
PMS1	0.02 (0.01)	0.08 (0.04)	0.05 (0.02)
pms1	0.08 (0.04)	0.12 (0.01)	0.09 (0.02)

[a] Transformation was carried out as described in Materials and Methods. Each entry is the mean of three determinations, with the standard error of the mean shown in parentheses. Half of each transformed culture was plated except for cases resulting in over 1,000 colonies per plate.

[b] Transformation with gapped pSDP21 normalized to uncut plasmid (1,000 to 5,000 transformants per 100 ng). *Msc*I digestion gives a 326-bp gap (see Fig. 1).

[c] Transformation with pNK2004-Z cut in *HIS4* normalized to pNK2004-Z cut in *URA3* (50 to 600 transformants per 100 ng). Uncut plasmid averaged fewer than one transformant per 100 ng. *Bgl*II digestion gives a single cut, and *Bst*XI gives a 390-bp gap (see Fig. 1).

with mutant *HIS4* genes at their normal positions. The four strains used were transformed by uncut episomal plasmid at about the same frequency. The nucleotide sequence divergence between *S. cerevisiae* and *S. carlsbergensis HIS4* genes over the first 1.6 kb is 16% (35a).

Initial selection of transformants was for the intact plasmid-borne yeast gene. For plasmid DNA cut in *HIS4*, Ura^+ was selected, and for plasmid DNA cut in *URA3*, His^+ was selected (plasmid integration control). This would allow recovery of transformants in which recombination gave a mutant gene or interfered with plasmid establishment.

The DSGs were introduced into episomal plasmids with a restriction endonuclease that left blunt ends. This greatly reduced self-ligation, which can occur with overlapping ends as frequently as recombinational repair, even for homologous DNAs (data not shown). Also, for homeologous DNA, gap filling would be unequivocal proof of recombinational repair.

Transformation frequency as a reflection of recombination efficiency. A DSB or DSG can induce recombination, both crossing over and gene conversion, when the DNAs are diverged (Table 1). For recipients with CHRIII*carl*, 85 to 90% of the transformants were His^+, showing that transformant recovery depended upon homology-driven recombinational interactions. As described below, ligation accounts for most of the His^- transformants with episomal plasmids.

DNA divergence inhibited recovery of transformants. Transformation with linear (i.e., gapped) episomal plasmid was normalized to that with the uncut episomal plasmid. For all transformants examined, episomal plasmid could be isolated. Relative to transformation of recipients having CHRIII*cere* (Table 1), the transformation frequency for CHRIII*carl* recipients was reduced 30-fold in Pms^+ recipients and approximately 10-fold in Pms^- recipients. For integrative plasmids, the transformation frequency for plasmid cut in *HIS4* was normalized to transformation with plasmid cut in *URA3*. The relative transformation frequency is greater than 1 for the homologous recombination control, presumably because the Ty insertion of the chromosomal *ura3-52* allele interferes with plasmid integration at *ura3*. Transformation with linear integrative plasmid was reduced 35- to 80-fold in Pms^+ recipients and 15- to 40-fold in Pms^- recipients. Thus, crossing over and nonreciprocal recombination events are reduced in response to 16% sequence divergence by a comparable factor, perhaps with crossing over being more sensitive.

Sequence divergence seemed to have no effect on the frequency of gap filling as compared with integration. Linear episomal pNK2004 gave a fivefold-higher relative transformation frequency than linear integrative pNK2004-Z plasmid for recipients containing either CHRIII*cere* or CHRIII*carl* (data not shown). Plasmid DNAs were digested with *Bst*XI, which makes a 390-bp gap within *HIS4cere* (Fig. 1), and their transformation frequencies were normalized to that for uncut episomal plasmid. Chromosomal insertions of pNK2004 are stable in the recipient strains (35b). The relative difference in transformation efficiency between episomal and integrating plasmids may reflect the likelihood of gene conversion with or without crossing over, different mechanisms of recombination (see Discussion), and/or the plasmids used.

Compared with the inefficiency of homeologous recombination in bacteria (37), the homeology-dependent reduction in transformation frequency in yeast cells is quite modest. The relative transformation frequencies for the CHRIII*carl* recipients with both episomal and integrative plasmids (Table 1) appear to be two- to threefold higher with Pms^- recipients than with Pms^+ recipients. However, the variability of the measurements (see standard errors) indicates that the differences may not be significant. Thus, DNA mismatch repair associated with *PMS1* appears to have little or no effect on the overall capability for recombination between homeologous DNAs in yeast cells.

Gene conversion tracts in episomal plasmids following recombination between homeologous DNA duplexes. The following features of plasmid chromosome recombination were examined: gene conversion of one or both preexisting plasmid ends along with the gap filling, continuity, and length of the gene conversion tracts and the presence of more than one type of plasmid within a transformant. The products of recombination between the two homeologous *HIS4* regions were all examined for restriction site polymorphisms (Fig. 1), and nucleotide sequence information was subsequently obtained for many of them (see Fig. 3).

Since gene conversion is the nonreciprocal transfer of genetic information between homologous (or homeologous) DNA duplexes, we consider filling of a gap in a plasmid as gene conversion, regardless of associated events at the ends of the plasmid DNA used. Associated with the gap filling, there can be transfer of information to either one or both strands of a recipient duplex end, referred to as one- and two-strand gene conversion. In meiosis, two-strand gene conversion has generally been explained as the long-patch repair of DNA mispairs present in heteroduplex DNA (hDNA) formed during recombination (33). It might also arise by nucleolytic expansion of a DSB to a gap prior to recombinational interactions which include gap filling (52) or a template switch during recombination-dependent DNA synthesis. One-strand gene conversion, indicative of the formation of hDNA, generates two genotypically distinct products from a single event. Examples include postmeiotic segregation (33) and postswitching segregation (36) in yeast cells. One-strand and two-strand gene conversion can be distinguished only if all products of recombination are examined.

The identification of two genotypically different cell lines in association with a single recombination event is taken as direct evidence of the formation of stable hDNA. If the initial recombinant plasmid carried a region of hDNA that was resolved by replication, two types of plasmid would result. To recover representatives from mixed plasmid populations within

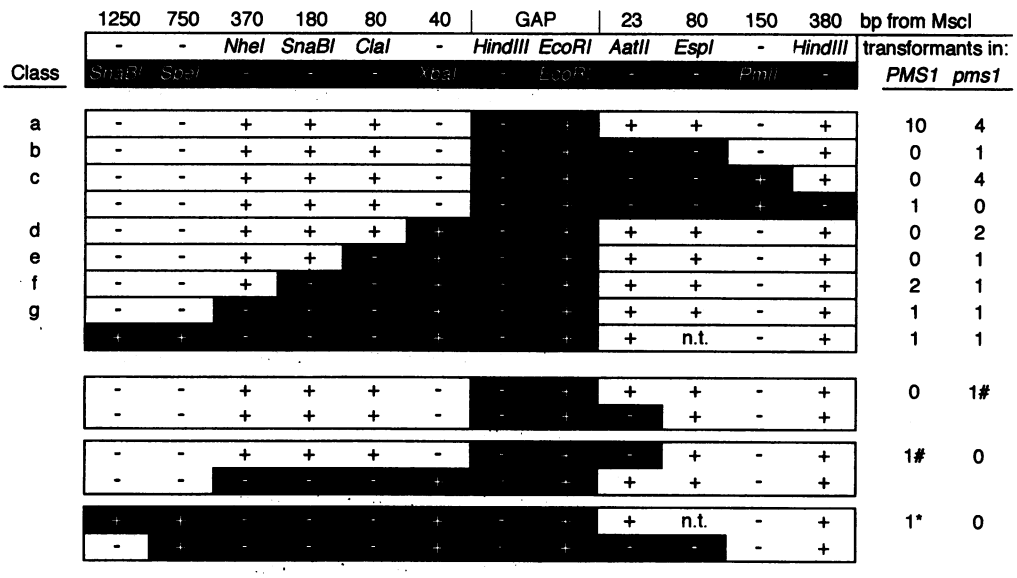

FIG. 2. Restriction maps for the *HIS4* regions of recombinant plasmids isolated from transformants derived from strains with CHRIII*carl*. Mixed plasmids: #, *HIS4* regions present on a heterodimeric plasmid; *, *HIS4* regions from two plasmids isolated from the same transformant colony. n.t., not tested. Class designations refer to Fig. 3B. □, *HIS4cere*; ■, *HIS4carl*.

individual yeast transformants, plasmid DNA was isolated from a culture inoculated with the entire transformant colony and used to transform *E. coli* (see Materials and Methods). The plasmid DNA used for restriction analysis was isolated from a culture inoculated with at least 15 *E. coli* transformant colonies.

The plasmid DNA used for transformation contained a DSG created by *Msc*I, resulting in a 326-bp gap in *HIS4cere* (Fig. 1). Plasmid DNA from 37 transformants of the *PMS1* and *pms1* strains was analyzed. Among these transformants, four carried nonrecombinant plasmids with a deletion corresponding to loss of the *Msc*I fragment, and an *Msc*I site was generated in three of these, presumably the result of self-ligation. The remaining 33 transformants carried recombinant plasmids, whose restriction patterns are shown in Fig. 2; 32 of these transformants were His^+. For the region of each plasmid corresponding to the *Msc*I fragment of *HIS4cere*, no deletions were detected, the *Eco*RI site common to both *HIS4* genes was present, and the *Hin*dIII site present within the *Msc*I fragment of *HIS4cere* was absent. Thus, the gap had been accurately filled by recombinational repair using the chromosomal *HIS4carl* DNA. Several of these plasmids had gene conversion tracts (indicated in black) extending from the filled gap, and in all cases the combined tracts were continuous (i.e., no patches) at the level of resolution by restriction analysis (Fig. 2). Nucleotide sequences extending at least 300 bp to each side of the gap were obtained for 23 of the plasmids (Fig. 3), and the results confirmed the continuity of the tracts. Surprisingly, only one transformant of the *pms1* recipient that carried a mixed plasmid population was obtained, contrary to results with homologous plasmids (see below).

In 30 of the 33 transformants carrying recombinant plasmids, the cells of a given transformant colony contained a single monomeric plasmid (Fig. 2). In 14 of these 30, corresponding to 28 of 60 plasmid ends, conversion tracts were apparently limited to gap filling. The tracts did not extend either to 40 bp from the left (*Xba*I in *HIS4carl*) or to 23 bp from the right (*Aat*II site in *HIS4cere*) of the original break points. The remaining 16 transformants carried plasmids with two-strand gene conversion tracts associated with the filled gap that extended either from the left or the right, but not in both directions. This corresponds to 16 of the 60 ends being converted. For the Pms^+ strain, 5 among 30 ends were converted, whereas for the Pms^- recipient, 11 among 32 ends were converted. As this difference is not statistically significant, substantially higher numbers of transformants would need to be examined to establish whether the level of conversion depends on *PMS1*.

On the basis of restriction analysis, at least one of the ends generated by the DSB appeared to be preserved during recombination between homeologous DNAs. Of the aforementioned 30 recombinant plasmids, conversion tracts could have extended from 60 *Msc*I-generated ends of input plasmid DNA. For 44 of the ends, either it was preserved or conversion did not extend beyond 40 bp from the gap. The first base pair difference between the homeologous *HIS4* regions to the left of the gap is 9 bp from the *Msc*I site of *HIS4cere* and is within a *Hpa*II site of *HIS4carl* (Fig. 3A). Fifteen of the twenty plasmids that appeared to retain the parental plasmid sequences to the left side (i.e., they lacked the *Xba*I site of *HIS4carl*) also lacked the *Hpa*II site and therefore appeared to have undergone no gene conversion to the left side.

Twenty-three of the thirty plasmids were sequenced (summarized in Fig. 3). All junctions between *HIS4cere* and *HIS4carl* sequences occurred in regions of continuous sequence identity (homologous segments) of at least 5 bp. Of the six plasmids which carried the *Hpa*II site but not the *Xba*I site of *HIS4carl*, three had conversion tracts ending in the next homologous segment, L2. Of the remaining three plasmids, conversion tracts extended into L3 in one and L4 in two. In contrast to the left end, the first 8 bp of the right end had been converted in all 18 plasmids sequenced that by restriction analysis had shown no conversion to the right; i.e., they retained the *Aat*II site of *HIS4cere* (Fig. 3). While the first 8 bp on the left end are identical, the right end terminates with a 4-bp homologous segment followed by a 4-bp heterology (Fig. 3A).

For the 16 plasmids in which conversion tracts had extended

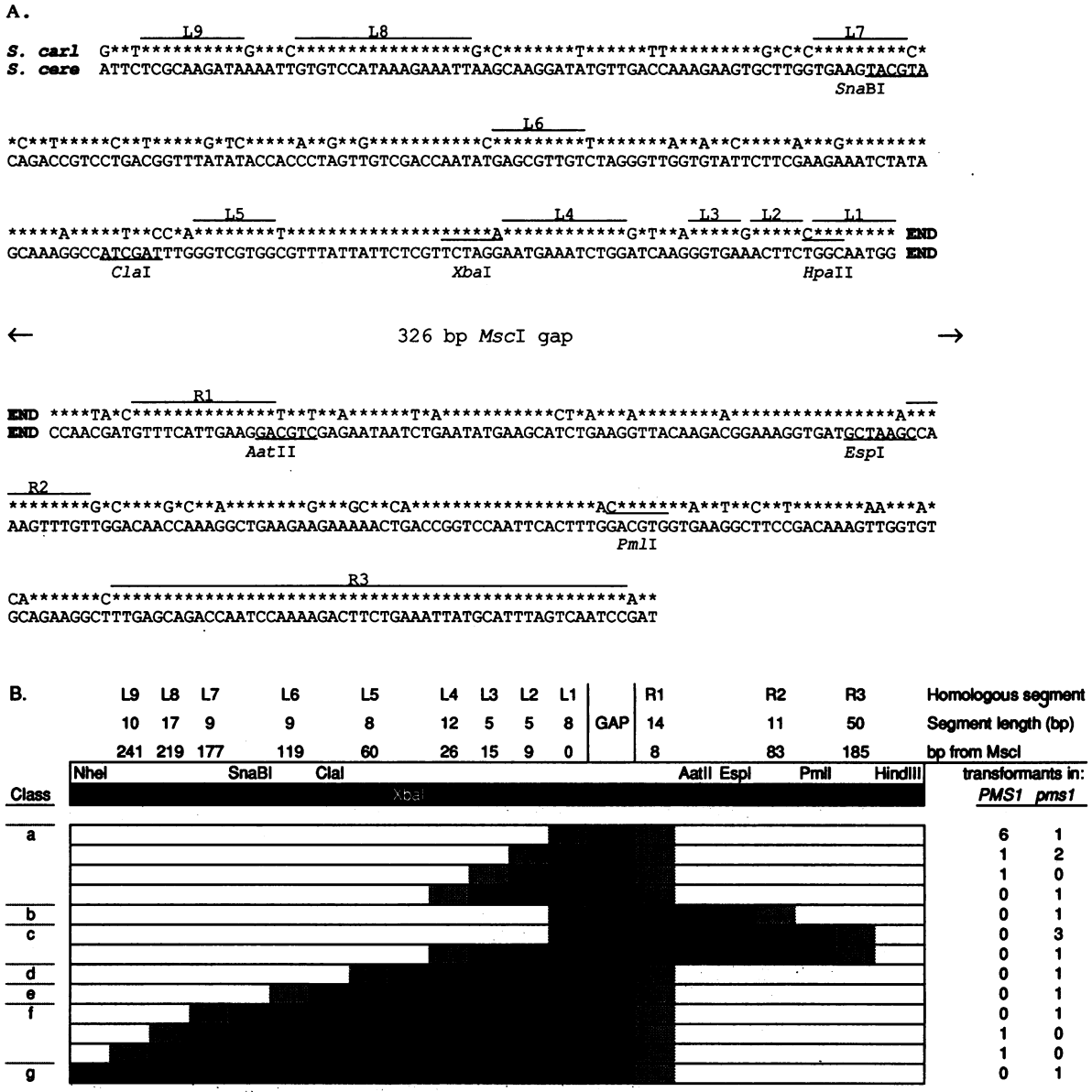

FIG. 3. Nucleotide sequence analysis of recombinant plasmids. (A) The nucleotide sequence for *HIS4carl* (top line in each row) is compared with the corresponding sequence of *HIS4cere* from bp 1685 to 1939 (top half) and from bp 2266 to 2503 (10). The region from 1940 to 2265 (not shown) corresponds to the gap generated in *HIS4cere* by *Msc*I digestion. Identical nucleotides are indicated by asterisks. END indicates the ends of the plasmid resulting from digestion by *Msc*I. Conversion tract endpoints were localized to regions of sequence identity (homologous segments) labeled L1 to L9 and R1 to R3. (B) Maps of conversion tracts for recombinant plasmids based on nucleotide sequences. Classes a through g correspond to those in Fig. 2. *HIS4cere* sequences are shown in white, and *HIS4carl* sequences are shown in black. The homologous segments comprising the junctions between *HIS4cere* and *HIS4carl* sequences are indicated in gray to reflect the sequence identity; the distances between the segments and the nearest *Msc*I cut site are given. The nucleotide sequence for the class g plasmid was obtained for the first 370 bp left of the gap.

at least 40 bp from the gap, most of the tracts were significantly longer. Twelve of the tracts extended >150 bp, and among, these five extended >370 bp. The median length of the conversion tract extension for the 16 plasmids was 185 bp, which corresponds to the distance between the gap and the homologous segment, R3.

The three transformants omitted from the foregoing analysis correspond to the mixed transformants in Fig. 2. The cells in two of these transformants carried head-to-tail heterodimeric plasmids. In one, the two copies of *HIS4* had conversion tracts that extended in opposite directions from the filled gap; in the other, one copy of *HIS4* had a conversion tract extending right. These plasmid could represent a cointegrate of two recombinant plasmids formed independently or, the more intriguing possibility, a plasmid formed during a single, complex recombination event. The third transformant gave rise to cells that collectively carried two different plasmids. One plasmid had a conversion tract extending to the left of the filled gap, and the other had tracts extending in both directions from the filled gap. These plasmids could have arisen by independent trans-

TABLE 2. Gene conversion of plasmid ends in association with gap filling

Relevant genotypes of recipient strains	No. with conversion[a]					
	−,−	Mixed −,− and +,− or −,− and −,+	+,− or −,+	Mixed +,− and −,+	Mixed +,− and +,+ or −,+ and +,+	+,+
his4cere						
PMS1	1	1	4	0	2	12
pms1	4	5	7	4	2	0
his4carI[b]						
PMS1	10	1[c]	5	0	1	0
pms1	7	0	9	0	0	0

[a] The parental plasmid carries *his4cere*. + or −, conversion or no conversion of the plasmid allele at sites 80 bp from the left end (first symbol) or right end (second symbol) of the gap (see text); mixed, isolation of two plasmids from a single transformant.
[b] Results taken from Fig. 2. Conversion tracts that did not extend 80 bp were classified as no conversion for the purpose of this comparison.
[c] Present as a heterodimeric plasmid.

formation events or by segregation of genotypically different strands present on the initial recombinant plasmid molecule. If the latter, the recombination event involved regions, from left to right, of one-strand gene conversion, two-strand gene conversion, gap filling, and one-strand gene conversion (Fig. 2).

Gene conversion tracts in episomal plasmids following recombination between homologous DNAs. Homologous and homeologous recombination were compared in terms of the fate of plasmid ends and the role of *PMS1*. Recombination between homologous DNAs differed significantly from that for homeologous DNAs in terms of conversion of the ends and the role of the *PMS1* gene.

To monitor the conversion events, the *Cla*I and *Esp*I sites in *HIS4cere*, located 80 bp to either side of the *Msc*I fragment, were mutated in order to provide markers (two- or three-base inserts, respectively) in pSDP29. Recipients carrying CHRII-I*cere* were transformed with pSDP29 gapped with *Msc*I. Plasmid populations were isolated from the transformants and analyzed as described above. The results are shown in Tables 2 and 3. Included for comparison are the results for the homeologous DNAs describing conversion only at the *Cla*I and *Esp*I sites of the plasmid-borne *HIS4cere* (taken from Fig. 2). For this comparison, conversion tracts that do not extend to these sites are classified as no conversion. The columns in Table 2 proceeding from left to right correspond to the following categories of gene conversion: no gene conversion, one-strand gene conversion to one side, two-strand gene conversion to one side, one-strand gene conversion to both sides, one-strand gene conversion to one side with two-strand gene conversion to the other side, and two-strand gene conversion to both sides. For the purpose of discussion, this interpretation assumes that transformants carrying a mixture of recombinant plasmids arose from a single transformation event.

TABLE 3. Fate of individual plasmid ends after transformation

Conversion type	% of total events[a]			
	Homologous		Homeologous	
	PMS1	*pms1*	*PMS1*	*pms1*
None or restoration	18 (7)	46 (20)	76 (26)	72 (23)
1-strand (hDNA)	7.5 (3)	34 (15)	6 (2)	0 (0)
2-strand	75 (30)	20 (9)	18 (6)	28 (9)
Plasmids with 2-strand conversion at both ends				
Observed[b]	60 (12)	0 (0)	0 (0)	0 (0)
Expected[c]	56 (11)	4 (1)	3 (>1)	8 (1)

[a] Results from Table 2 shown with respect to the individual ends given as percentage of total events. Observed (or expected) absolute number of events is shown in parentheses.
[b] Results from last column of Table 2 given as percent of total number of plasmids.
[c] Calculated from the observed frequency of two-strand conversion of an individual end.

For homologous DNAs, one difference between the results for the Pms$^+$ and Pms$^-$ recipients is immediately apparent (Table 2). Most plasmids recovered from transformants of the Pms$^+$ strain (60%) had undergone two-strand gene conversion in both directions, while there were no such plasmids recovered from the Pms$^-$ strain. Other differences are revealed when the results are shown in terms of the individual ends of the transforming plasmid DNA (Table 3). A fivefold-higher frequency of one-strand conversion was observed for the Pms$^-$ strain ($P \leq 0.005$ for one-strand conversion versus restoration or no conversion and two-strand conversion). (Therefore, the frequency of transformants containing a mixture of plasmids with the Pms$^+$ recipient may approximate the upper limit for the frequency of multiple transformation events.) Also, total gene conversion was reduced in the Pms$^-$ strain ($P \leq 0.011$ for no conversion [or restoration] versus one-strand and two-strand conversion). A 4:1 bias in favor of two-strand conversion versus restoration was observed for the Pms$^+$ strain; this bias deviates significantly from 1:1 ($P \leq 0.014$). The 2:1 bias in favor of restoration observed for the Pms$^-$ strain does not deviate significantly from 1:1. Thus, the Pms$^-$ mutation leads to an expected increase in the preservation of hDNA formed during recombination, i.e., one-strand conversion, but total conversion is reduced.

Recombination between homeologous DNAs is considerably different from that for homologous DNAs (Tables 2 and 3). With homeologous DNAs, no significant difference in the conversion patterns between the Pms$^+$ and Pms$^-$ recipients was observed. Taking all transformants (with homeologous DNAs) together, one-strand plus two-strand conversion of the ends (extending at least 80 bp) is only 26%, which is significantly lower than the 54% observed for homologous DNAs in the Pms$^-$ recipient ($P \leq 0.004$). Of particular note is that few transformants carrying a mixture of plasmids were recovered, none of which were from the Pms$^-$ recipient. These results suggest that extensive hDNA either does not form or is transient, even in a *pms1* background (see Discussion).

Recombination between homeologous DNAs and between homologous DNAs in a Pms$^-$ background resulted in two-strand conversion at only 23 and 20%, respectively, of plasmid

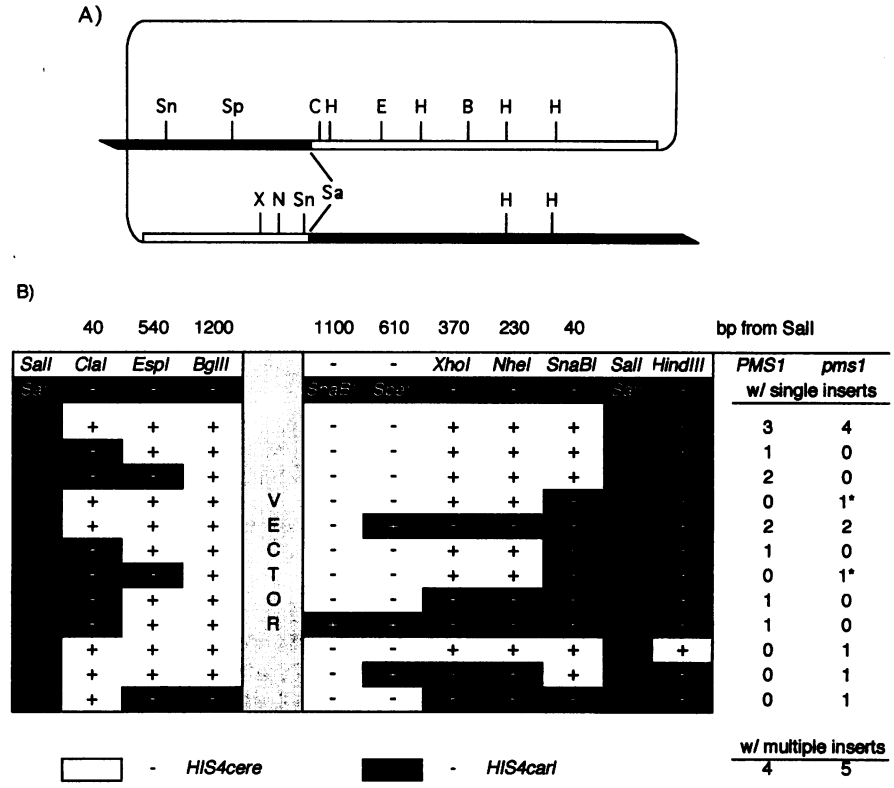

FIG. 4. Gene conversion tracts associated with integration of plasmids carrying *HIS4cere* into chromosomal *HIS4carl*. For transformation, YIpSDP26 was linearized in *HIS4cere* with *Sal*I, which is common to both *HIS4* regions. The presence or absence of restriction sites was determined by Southern blotting (see Materials and Methods). The DNA probe shares homology with the vector part of the integrated plasmid. (A) Structure expected for precise integration of plasmid into the chromosomal *Sal*I site, i.e., no associated gene conversion. B, *Bgl*II; C, *Cla*I; E, *Esp*I; H, *Hin*dIII; N, *Nhe*I; Sa, *Sal*I; Sn, *Sna*BI; Sp, *Spe*I; and X, *Xho*I. (B) Results of Southern analysis. 1*, one-strand conversion at *Sna*BI site (see text).

ends. Also, none of the plasmids had undergone two-strand conversion in both directions from the gap. The high frequency of two-strand conversion, often in both directions, observed for homologous DNAs clearly depended on a functional *PMS1* gene. From these results, we conclude that exonucleolytic expansion of a DSB to a DSG prior to recombination (52) cannot account for the two-strand gene conversion (see Discussion).

Gene conversion of plasmid-borne sequences associated with plasmid integration. A nonreplicating plasmid carrying yeast DNA can integrate into the chromosomal homolog by crossing over. Reciprocal exchange is often associated with gene conversion between homologous DNAs (summarized in reference 33). To examine this for diverged DNAs, the fate of plasmid DNA carrying *HIS4cere* was monitored after integration at the chromosomal *HIS4carl*. The integration results in two directly repeated copies of the *HIS4* region flanking the remainder of the plasmid DNA, where each copy should be a composite of the original chromosomal and plasmid-borne copies (Fig. 4). Integration was frequently associated with conversion of one or both ends.

A DSB was introduced into the integrative plasmid pSDP26 (containing *HIS4cere*) with *Sal*I, which is present in both *HIS4cere* and *HIS4carl*. The plasmid was transformed into *PMS1* and *pms1*Δ recipients carrying CHRIII*carl*. Genomic DNA was isolated from the transformants and digested with appropriate combinations of restriction endonucleases for Southern hybridization analysis (Materials and Methods). The probe was specific to the non-yeast vector part of pSDP26. The information obtained in this analysis was limited primarily to the fate of plasmid-borne *HIS4cere* sequences and to transformants containing a single insert (Fig. 4). Multiple plasmid insertion could be detected from *Hin*dIII digests, because of the additional sites present in *HIS4cere* (Fig. 1). The fate of the ends of the transforming linear plasmid DNA could be determined to within 50 bp, the distance between the *Cla*I and *Sna*BI sites and the ends.

Among 11 transformants of the Pms$^+$ recipient, half (11 of 22) of the ends were converted. Conversion at only one end was observed in five transformants, and conversion at both ends was observed in three transformants. While the number of transformants analyzed was limited, this clearly differs from the pattern of conversion for episomal plasmids, in which case only 18% of the ends were converted and there were no bidirectional events (Table 3). Conversion tracts, when present, were continuous and extended inward from the original end of the transforming DNA, and only one type of recombinant structure was observed for the cells of a given transformant colony (Fig. 4).

Whereas the results with episomal plasmid were comparable for the Pms$^+$ and Pms$^-$ strains, there appear to be differences when plasmids are integrated. Six of eleven transformants of the Pms$^-$ strain were similar to those seen with the Pms$^+$ strain; four showed no conversion, and two showed two-strand conversion to only one side. For two of the remaining five transformants (with a single insert), about half of the cells had retained the *HIS4cere* *Sna*BI site and half had lost it, as estimated from the blot (not shown). Thus, the ends had

undergone one-strand conversion. Two transformants exhibited patches of conversion, and one exhibited conversion in sequences adjacent to the plasmid insert.

DISCUSSION

We have used a plasmid model system to examine the consequences of nucleotide sequence divergence on recombination associated with the repair of defined double-strand gaps or breaks in DNA. Previous reports (3, 15, 23, 29) have concentrated on the consequences of undefined lesions or spontaneous recombination. In this study, the role of DNA mismatch repair was examined for cases when few versus many mispairs might form during recombination. Possible differences in mechanisms of recombination between homologous versus diverged DNAs were identified.

Gaps in episomal plasmid DNA can be efficiently repaired during transformation through recombinational interactions with homeologous as well as homologous chromosomal DNA. A 16% reduction in homology reduced gap filling only 10- to 30-fold (Table 1), consistent with previous findings for spontaneous and DSB-induced gene conversion, for which the levels are typically 10- to 100-fold lower (3, 15, 21, 23, 29). Plasmid integration was slightly more sensitive to reduced homology, whereas meiotic reciprocal exchange is reduced at least 100-fold (29–31, 35b). These effects of divergence are much less than expected on the basis of results for recombination between *E. coli* and *Salmonella* DNAs (37) or between diverged DNAs in *E. coli* (34), suggesting that yeast cells are much more tolerant of mismatches during spontaneous and DSB-induced recombination. The present results are consistent with our previous observations of radiation-induced recombination between diverged chromosomes (41).

Gene conversion of homologous DNAs and the role of DNA mismatch repair. The fate of transforming plasmids having gaps in regions that were homologous to chromosomal DNA was examined to evaluate the potential for recombinational repair and the role of mismatch repair functions. The results for the *pms1* mutant (Table 3) clearly show that hDNA is a common intermediate in DSB repair, leading to one-strand gene conversion in the absence of *PMS1*. The major recombinational repair category for a Pms^+ recipient was two-strand gene conversion extending at least 80 bp from an end (Table 2). The strong bias in favor of two-strand conversion over restoration was dependent upon *PMS1*, which cannot be explained as nucleolytic gap expansion prior to recombination (52), as discussed below. Rather, most of the two-strand conversion appears to result from mismatch repair.

Mismatch repair of hDNA recombination intermediates in *S. cerevisiae* is thought to occur without strand bias (33); i.e., the hDNA is either converted (two-strand) to a recombinant genotype or restored to the parental genotype with equal probability. This hypothesis is inconsistent with the results described above, which showed that there is a significant bias favoring conversion (Tables 2 and 3).

The excess of two-strand conversions observed might result from mismatch repair directed preferentially to the strand of hDNA with an end generated by a DSB. In bacteria, mismatch repair during replication or recombination is directed to the strand with a preexisting end or, in the case of *E. coli*, an end generated by the MutH protein (8, 25). Also, the proximity between a mispair and a strand break affects repair efficiency (25). Since *PMS1* is homologous to bacterial mismatch repair genes (20) and is involved in replication fidelity (56), strand discontinuities might direct mismatch repair in yeast cells. The bias that we observed might be allele specific; however, it would have to be true for both alleles, which would form frameshift mispairs. In bacteria, repair of frameshift mispairs is strand specific, not allele specific (8, 22).

There are other implications of strand bias in mismatch repair during recombination. For example, there may be additional sources of strand breaks, such as might occur during resolution of Holliday structures. Also, the proximity between a mispair and a given break might affect mismatch repair efficiency in *S. cerevisiae* as in bacteria. These implications when applied to current models for DSB-induced recombination (39, 53) could provide an explanation for conversion gradients during meiosis.

Related findings have been reported for meiotic recombination at the yeast *ARG4* locus, which appears to be initiated by a site-specific DSB (28, 50). Most gene conversion at *ARG4* seems to involve formation of extensive hDNA and mismatch repair resulting in two-strand conversion (summarized in reference 51). Gene conversion usually leads to a transfer of genetic information to the chromosome on which the initiation event occurred (28). For markers located a few hundred base pairs to each side of the initiation site, 64% of conversion events for one of the markers included conversion of the marker on the other side (44).

In the absence of the *PMS1* gene product, two-strand conversion events were still detected, and the number of converted sites (one-strand plus two-strand) decreased (Table 2B). The two-strand conversion events might have arisen by *PMS1*-independent mismatch repair, possibly involving other recently identified mismatch repair genes (27, 38) or by a conversion mechanism not involving mismatch repair (discussed below). The decrease in conversion might be due to a relative increase in mismatch repair leading to restoration; a bias in favor of restoration was observed, but did not deviate significantly from 1:1. Alternatively, the absence of the PMS1 protein might affect the formation or persistence of hDNA.

Gene conversion does not appear to involve nucleolytic gap expansion prior to recombination. The nucleolytic expansion of a DSB or DSG to a larger DSG before any recombinational interactions, i.e., gap expansion, could account for gene conversion (52). This should lead mostly to bidirectional two-strand conversion from the original ends regardless of homology. However, following double-strand cleavage by the HO endonuclease at the *MAT* locus, little or no double-strand nucleolytic degradation occurs at the ends prior to recombination (42). A variety of evidence suggests that meiotic recombination at *ARG4* involves formation of extensive hDNA adjacent to a DSB (summarized in reference 51).

Although we observed an excess of bidirectional two-strand conversion for homologous DNAs during gap repair, it must have occurred after initiation of recombination since it depended upon *PMS1* (Table 2). For homeologous DNAs, nearly all the recombinant episomal plasmids retained the plasmid-borne DNA to within 26 bp of at least one side of the original gap (Fig. 2 and 3). Gap expansion to only one side (prior to recombination) might explain some two-strand conversion, especially for homeologous DNA and for homologous DNA in a *pms1* strain. However, a mechanism limiting double-strand degradation to one side seems unlikely.

Recombination repair of homeologous DNAs and the lack of mismatch repair. The pattern of gene conversion suggests that plasmid gap repair mechanisms differ between homologous and homeologous DNAs. First, conversion of the ends during repair of gaps using homeologous DNA was significantly less frequent (Table 2). Second, there was little difference in the frequencies or patterns of conversion (Fig. 2) between the

PMS1 and *pms1* recipient strains, and nearly all conversion was two-strand.

Acquisition of information from the chromosome was directional from the ends and continuous (Fig. 3). Except for the integrating plasmids in a Pms$^-$ strain, there were no conversion patches. These observations generally exclude mechanisms that would act randomly on mismatches. It is possible that some *PMS1*-independent mismatch repair occurs with homeologous DNA, as might be the case with homologous DNA (Table 3). Such repair should be less efficient since the *pms1* mutation increased one-strand conversion for homologous DNAs. However, the higher number of mispairs (increased substrate concentration) in hDNA formed from homeologous DNAs might counteract lower efficiency.

The comparable responses between Pms$^+$ and Pms$^-$ strains and the lack of one-strand conversion events (Table 3) alternatively suggest that little or no mismatch repair occurs during recombination between homeologous DNAs. The *PMS1* independence in recombination cannot be explained as a saturation of mismatch repair in the wild type by a high concentration of mismatches present in hDNA, as has been observed during chromosomal transformation of *Streptococcus pneumoniae* (8, 14). If there were saturation, mixtures of recombinant plasmids within the transformants would be expected, contrary to observation.

We suggest that there may be a failure to form an appropriate substrate for mismatch repair (discussed below). The hDNA tracts formed may be very short, perhaps not including base mispairs, or alternatively, extensive tracts may form but exist too briefly for efficient recognition of base mispairs. If true, a mechanism not involving mismatch repair would be required to explain the gene conversion seen.

Most of the observed conversion tracts were short, >10 bp (Fig. 3B). The left end was completely preserved in half (15 of 30) of the plasmids. Of 18 plasmids that had not been converted past 23 bp to the right of the gap, all had conversion tracts extending into the R1 homologous segment (Fig. 3). The first 8 bp left of the gap are homologous (L1), while to the right the first 4 bp are homologous and the next 4 are heterologous (3 of 4), followed by the R1 homologous segment. We suggest that the frequent conversion of the 8 bp to the right is due to the 4-bp heterology and interaction between plasmid and chromosomal DNAs, rather than to plasmid degradation prior to the interaction. These conversion tracts, and perhaps some tracts extending left of the gap into the homologous segments L2, L3, and L4, might have resulted from 3′-to-5′ editing by a DNA polymerase prior to recombination-dependent DNA synthesis. Alternatively, *RAD1* has been implicated in the removal of heterologous sequences at ends formed by a DSB during recombinational repair (11). The contrast between events occurring at the left and right ends suggests that different mechanisms are responsible for the short- versus long-tract conversion events observed.

Reduced efficiency of recombinational repair between homeologous DNAs. The potential for recombinational repair between diverged DNAs might depend on homology over an extended region or on the lengths of segments of continuous homology. In *E. coli*, there appears to be a minimum length of homologous DNA below which recombination becomes inefficient (45), termed the minimum efficient processing segment (MEPS). For recombination between homeologous DNA, the reciprocal recombination efficiency appears to correlate closely to the sum of recombination efficiencies for DNA segments of continuous homology (46). In *S. cerevisiae*, the frequency of gene conversion on a plasmid decreases 1,000-fold for a 10-fold decrease in length of a homologous donor segment, flanked by heterologous DNA, covering the marker (1). For spontaneous chromosome recombination involving direct repeats or ectopic recombination, the MEPS is approximately 250 bp (19). In our study, the longest stretches of unbroken homology between the *HIS4* genes within 300 bp left and 920 bp right of the *Msc*I-generated gap are 20 and 50 bp (R3), respectively (Fig. 3) (35a). Junctions between *HIS4cere* and *HIS4carl* sequences occurred in homologous segments of 5 to 50 bp, with a median length between 9 and 10 bp (Fig. 3). Based on estimates of the MEPS, the overall recombination efficiency for DNAs having stretches of homology averaging 6 to 7 bp might be expected to be much lower than we observed. A finding similar to ours has been reported for DSB-induced recombination between highly diverged DNAs during yeast transformation (23). Our results and those of Mezard et al. (23) suggest that either the MEPS for DSB-initiated recombination in yeast cells is much lower than for spontaneous recombination or recombination efficiency can be influenced by regional homology allowing pairing interactions but not necessarily hDNA formation. If the MEPS governs recombination, then in *S. cerevisiae*, it appears that for both spontaneous (3, 15, 21, 41) and DSB-induced (reference 23 and this report) recombination, the DNA does not have to be homologous.

Mismatch repair might be indirectly responsible for reduced efficiency of recombination between homeologous DNAs. Multiple heterologies may lead to secondary recombination events, resulting either from an expansion of hDNA and subsequent mismatch repair tracts or from DSBs formed by overlapping mismatch repair tracts. Introduction of heterologies on the order of 1 per 0.5 to 1 kb altered the types of recombination events observed for otherwise homologous DNAs, and the alterations were found to depend on *PMS1* (7). Repair of highly mismatched hDNA might lead to the loss of plasmid or chromosomal DNA. If so, *PMS1* does not play an important role. A *pms1* mutation led to at most a two- to threefold increase in transformation frequency (Table 1), which was not statistically significant. However, a comparable increase in spontaneous ectopic recombination has been reported for homologous as well as homeologous DNAs (3). Furthermore, recombination can be induced by a single-strand break within diverged repeats on a transforming plasmid without apparently affecting plasmid survival (21). Given that transformation efficiency was reduced only 10- to 30-fold and that the potential frequency of mismatches is high if hDNA is formed, other mismatch repair systems would also seem to play a minor role.

Processing of ends and implications for models of recombination. The fate of the two ends of a DSG depends on whether they are homologous or homeologous with the chromosomal copy (Table 2). We suggest that differences in classes of recombinants between homologous and homeologous DNAs and the reduced efficiency reflect the initial interaction between one end of a gapped plasmid molecule and the intact substrate. For homologous DNAs, the two ends appear to be processed similarly, the ends usually undergo conversion, and they are subject to mismatch repair directed by *PMS1* and/or some other system(s). For homeologous plasmid DNAs, there seems to be little *PMS1*-directed mismatch repair, and at most only one end undergoes extensive conversion.

The results with homologous and homeologous DNAs are consistent with the original model for DSB-induced recombination (39), features of which are incorporated in succeeding models (51, 52; discussed in reference 53). In general, the roles of the two ends in repair differ in that only one end is proposed to initiate the repair process with an intact homolog (or a

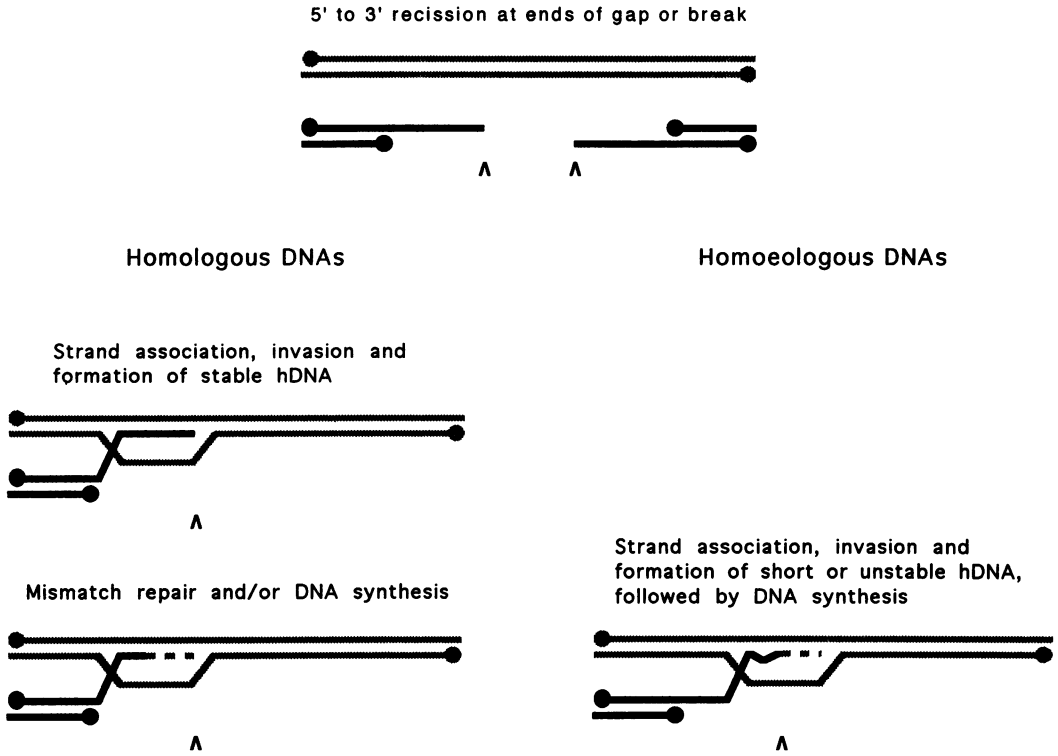

FIG. 5. Model for the early steps in recombinational repair of a DSG (or DSB) for both homologous and homeologous DNAs. The ends formed by the break (indicated by arrows) undergo 5′-to-3′ exonucleolytic processing (39). Parental genotypes are indicated in gray for the intact duplex and in black for the broken duplex. Parental strands are indicated by unbroken lines, and synthesized strands are indicated by dashed lines. The 5′ end of each strand is indicated with a ball.

homeolog). The participation of the second end depends on events associated with the processing of the first end. The second end need not interact with the intact chromosome for repair to be complete; it could interact with a newly synthesized strand extending from the initiating end (39). Only if both ends interact with the intact chromosome can crossing over occur.

We propose that the end of a DSB, such as that derived from a 5′-to-3′ exonucleolytic digestion (39), can interact with an intact molecule even when the DNAs are diverged (Fig. 5). Possibly the same or similar mechanisms that provide for the recognition and association of homologous DNAs, such as paranemic interactions (5), also exist for diverged DNAs. For homologous DNAs, stable hDNA can be formed and may be subject to *PMS1*-directed excision of the invading strand end, which could account for the conversion bias observed. The next step involves DNA synthesis.

DNA divergence both limits the recombinational interactions with the initiating end and alters the processing of the end as compared with homologous DNA. For example, the frequency of repair is reduced for homeologous plasmids, conversion is reduced during repair, and a *PMS1* mutation has little or no effect. We suggest that the capability to form extensive or stable hDNA during the interaction between the initiating end and the intact chromosome may be impaired. However, once DNA synthesis is initiated at the 3′ end, the resulting homoduplex could stabilize the interaction (Fig. 5). Such a failure to form stable or extensive hDNA could preclude either one-strand conversion or two-strand conversion due to mismatch repair.

In the original model (39), involvement of the second end in completion of repair could occur through one of two processes. The second end could interact with the unbroken chromosome, as also suggested in a later models (51, 52). Alternatively, the newly synthesized DNA (extending past the gap region) could interact directly with the second end. By the latter process, overlapping strands of homologous DNAs could form stable hDNA, which could then undergo mismatch repair directed to either strand (39). Bias in conversion at the second end might not be expected since both strands are discontinuous. For homeologous DNAs, interaction of the overlapping strands might be unstable. As for the first end, DNA synthesis would stabilize the interaction (except that in this case there are two 3′ ends to initiate synthesis) and the unstably paired 3′ end could then be degraded. If DNA synthesis were reinitiated with the newly synthesized gap-filled end, two-strand conversion would occur, similar to the template switch mechanism proposed for recombinational repair of UV damage in phage T4 (12). If this were the predominant conversion mechanism for homeologous DNAs, it could account for the approximately 25% (18 to 28%; Table 3) conversion observed.

The results with the integrating plasmids demonstrate that crossing over can be induced by a DSB within homeologous DNA. This is consistent with the recent report of Harris et al. (15) for spontaneous recombination and crossing over between diverged DNAs. Since the pattern of conversion of the ends for the limited sample examined differs from that for episomal plasmids, it is possible that interactions leading strictly to gap filling could differ from those for crossing over. However, such conclusions must be tempered since the integrating plasmid in this study had a DSB, whereas the episomal plasmid had a gap.

Conclusions and implications. During the repair of broken

DNA, homology-dependent interactions are clearly important in directing recombination for both homologous and homeologous DNAs. The homology of ends determines the effectiveness and pattern of DSB-initiated recombination. For homologous DNAs, we have shown that there is efficient mismatch repair of recombination intermediates leading to gene conversion. Much of the *PMS1*-directed conversion may occur with strand bias toward the incoming strand. While the formation of hDNA between homologous DNAs may be important for stabilizing intermediates, the present results suggest that other homology-dependent interactions may predominate with homeologous DNAs.

With this system, we have demonstrated that gapped plasmids can be used to recover information from related DNAs in chromosomes, and the accuracy of the information remains intact, as evidenced by the His^+ gap-fill products. Thus, this system can be used effectively to generate chimeric genes derived from evolutionary related sources. In addition, we have shown that a DSB can be used to efficiently target DNA to a related DNA sequence. This approach would be useful for identifying and mapping genes within a gene family. While not specifically demonstrated, fragmentation of human DNA carried on yeast artificial chromosomes could be a manifestation of recombination between diverged DNAs (32). It will be interesting to determine the limit of divergence that would enable interaction and the possible role of recently identified mismatch repair mutants in extending the capability for targeting.

The present results with interactions between transforming DNA containing a break or gap and chromosomes complement our recent studies of single-strand break- and DSB-initiated recombination in transforming plasmids containing direct repeats (21). Either type of break was effective in inducing recombination between repeats that were diverged by approximately 15%, demonstrating once again that DNA lesions can effectively induce recombination between diverged DNAs.

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