A Defect in Mismatch Repair in *Saccharomyces cerevisiae* Stimulates Ectopic Recombination Between Homeologous Genes by an Excision Repair Dependent Process

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**ABSTRACT**

Null mutations in three recombination and DNA repair genes were studied to determine their effects on mitotic recombination between the duplicate AdoMet (S-adenosylmethionine) synthetase genes (*SAM1* and *SAM2*) in *Saccharomyces cerevisiae*. *SAM1* and *SAM2*, located on chromosomes XII and IV, respectively, encode functionally equivalent although differentially regulated AdoMet synthetases. These similar but not identical (homeologous) genes are 83% homologous at the nucleotide level and this identity is limited solely to the coding regions of the genes. Single frameshift mutations were introduced into the 5′ end of *SAM1* and the 3′ end of *SAM2* by restriction site ablation. The sequences surrounding these mutations differ significantly in their degree of homology to the corresponding area of the other gene. Mitotic ectopic recombination between the mutant *sam* genes occurs at a rate of $8.4 \times 10^{-9}$ in a wild-type genetic background. Gene conversion of the marker within the region of greater sequence homology occurs 20-fold more frequently than conversion of the marker within the region of relative sequence diversity. The relative orientation of the two genes prevents the recovery of translocations. Mitotic recombination between the *sam* genes is completely dependent on the DNA repair and recombination gene *RAD52*. A mutation in *PMS1*, a mismatch repair gene, causes a 4.5-fold increase in the rate of ectopic recombination. *RAD1*, an excision repair gene, is involved in modulating the pattern of coconversion during recombination between the homeologous *sam* genes. These results suggest that interactions between mismatch repair, excision repair and recombinational repair functions are involved in determining the ectopic conversion frequency between the *sam* genes.

**GENETIC** recombination between repeated genes is believed to be a major mechanism governing the evolution of gene families as well as alterations of genome structure (Edelman and Gally 1970). Mitotic nonreciprocal recombination, or gene conversion, has been observed between repeated genes in the yeasts *Saccharomyces cerevisiae* (Ernst, Stewart and Sherman 1981; Scherer and Davis 1980) and *Schizosaccharomyces pombe* (Munz and Leupold 1981). Such recombination events can give rise to new alleles or restore sequences that have been changed by mutation. The research from several laboratories has shown that reciprocal recombination between repeated genes inserted on the same chromosome can give rise to deletions, duplications and inversions in mitotic cells (Szostak and Wu 1980; Jackson and Fink 1981; Willis and Klein 1987; Klein 1988a). Reciprocal translocations, the product of reciprocal recombination between properly oriented genes on different chromosomes, have been observed to arise mitotically in *Saccharomyces cerevisiae* (Mikus and Petes 1982; Potter, Winsor and Lacroute, 1982; Sugawara and Szostak 1983; Fasullo and Davis 1987) and *Schizosaccharomyces pombe* (Szostak et al. 1986). These observations suggest that mitotic recombination between dispersed repeated elements in yeast could potentially rearrange the genome at a significant frequency.

For all of the reasons cited above, an organism must control the level of recombination between dispersed repeated elements. Mutants defective in recombination between repeated genes would be useful for studying this process. Mutations in several DNA recombination and repair genes have been found to have effects on mitotic ectopic recombination in yeasts (Munz and Leupold 1979; Petes and Hill 1988; Klein 1988b). The pleiotropic recombination and double-strand-break repair mutation *rad52* has been shown to cause defects in mating type interconversion (Malone and Esposito 1980; Weiffenbach and Haber 1981) exchange between *d* elements (Winston et al. 1984; Rothstein, Helms and Rosenberg 1987), we as direct repeat recombination in *Saccharomyces cerevisiae* (Jackson and Fink 1981; Klein and Petes 1981; Willis and Klein 1987). The *rad52* mutants are also defective in mitotic heteroallelic, as
well as meiotic recombination (Prakash et al. 1980; Game et al. 1980). Several laboratories recently reported a synergistic interaction between rad52 and the excision repair mutation, rad1, on mitotic direct repeat recombination (Schiestl and Prakash 1988; Klein 1988a; Thomas and Rothstein 1989). While both single mutations conferred small reductions in the frequency of ectopic recombination between directly repeated genes, the double mutant exhibited a manyfold greater reduction in frequency.

Here we describe an assay that utilizes the duplicate AdoMet synthetase genes, SAM1 and SAM2 from Saccharomyces cerevisiae. The SAM genes are located on different chromosomes (XII and IV, respectively; N. Rosenberg and R. Rothstein, unpublished data) and are 83% homologous at the DNA level (Thomas et al. 1988). The isozymes are 98% homologous at the amino acid level. Only a single SAM gene is required to obtain wild type growth on AdoMet-free medium. These genes were used in order to determine the role that homology plays in recombination between repeated genes at non-allelic (ectopic) positions. Mitotic ectopic recombination between the similar but not identical (homeologous; Carpenter 1984) sam genes occurs at a rate that is 8–23-fold lower than the rates reported for ectopic recombination between artificially repeated homologous genes (Jinks-Robertson and Pette 1986; Lichten and Haber 1989). The orientation of the sam genes relative to their centromeres prevents the recovery of reciprocal translocations and, therefore, limits the recombination events to gene conversion and rare double reciprocal exchanges. The conversion events we observed occurred more frequently between regions of greater homology.

We examined the effect of null mutations in several DNA repair and recombination genes on homologous ectopic recombination in mitotic cells. The pleiotropic double-strand-break repair and recombination mutation rad52 was chosen because of its profound effect on mitotic recombination (Game et al. 1980; Prakash et al. 1980; Malone and Esposito 1980; Jackson and Fink 1981; Rothstein, Helms and Rosenberg 1987; Willis and Klein 1987). There was no detectable recombination between the sam genes in rad52 mutant cells. Our analysis also included an investigation into the effect of the excision repair mutation rad1 because of its synergistic effect on direct and inverted repeat recombination when acting in conjunction with rad52 (Schiestl and Prakash 1988; Klein 1988a; Thomas and Rothstein 1989). Although synergy between rad1 and rad52 was not observed in our assay, the pattern of cointeraction in rad1 single mutants was different from wild type, similar to that observed after recombination between inverted repeats (Aguilera and Klein 1989). The effect of the mismatch repair mutation pms1 on recombination between the sam genes was of particular interest because they are extensively mismatched. The pms1 gene shares some DNA sequence homology with the mismatch repair genes mutL from Salmonella typhimurium, and heb from Streptococcus pneumoniae although the role of PMS1 in mismatch repair is not known (Kramer et al. 1989b). The rate of recombination between the homeologous sam genes in pms1 cells increased the same amount as recombination between homologous genes. This increase was dependent upon the RAD1 gene. These and other epistatic relationships suggest that the recombination events detected in this assay are the result of a complex interweaving of DNA repair pathways.

MATERIALS AND METHODS

Strains: All yeast strains used in this investigation were derived from W303-1A or W303-1B (Thomas and Rothstein 1989) and are listed in Table 1. Standard methods for mating, diploid selection, sporulation and tetrads dissection were employed (Sherman, Fink and Hicks 1986).

Escherichia coli strain SF8 was used as the host for all plasmids (Cameron et al. 1975).

Media: All Saccharomyces cerevisiae strains were maintained on YPD (2% peptone, 2% dextrose, 1% yeast extract) either in the presence or absence of AdoMet (Sigma). AdoMet at a concentration of 10 mg/ml was added to YPD plates or liquid medium to make a final concentration of 0.1 mg/ml. Single drop-out media used for selection of diploids and for tetrads analysis were made as described previously (Sherman, Fink and Hicks 1986), and modified by the addition of 60 µg/ml L-leucine.

Plasmids: All of the plasmids constructed for this study are listed in Table 2. The majority of the plasmids used in this study were derived from pWJ355 and pWJ356. pWJ355 was constructed by cloning a 1.8-kb Xhol/HindIII DNA fragment bearing the SAM2 gene into the SalI and HindIII sites in the polylinker of pGEM1 (Promega Biotec). pWJ391 was constructed by cloning a 2.5-kb EcoRI/HindIII fragment containing the SAM2 gene with additional 3' flanking DNA into pGEM2, replacing the polylinker. pWJ356 was constructed by cloning a 1.7-kb Xhol/SnaBI fragment containing the SAM1 gene into the SalI and Smal sites in the polylinker of pGEM1. All SAM sequences were obtained from the original clones of SAM1 and SAM2 (Thomas and Surdin-Kerjan 1987; Thomas et al. 1988).

Construction of sam mutations: Several deletion-disruption mutations were constructed for use in this study. pWJ356 was cut with BglIII within the SAM1 coding region (Figure 1A), flushed with Klenow (Boehringer-Mannheim), cut at the unique SalI site and then ligated to a 2.0-kb Hpal/SalI LEU2 fragment. This resulted in a construction in which 704 bp of SAM1 coding sequence is replaced with the LEU2 gene. After introduction of this disruption into yeast (see below), the sam1::LEU2 allele is created. Similarly, sam2::HIS3 was made by cutting pWJ355 at BglII, which lies 5' to the translational start codon of SAM2, and at the unique SalI site within the SAM2 coding sequence before ligating in the 1.3-kb BamHI/XhoI HIS3 sequence. In this disruption, the entire 5' end of the gene is replaced with HIS3 leaving only the 3' end of the SAM2 gene. To make the sam2::LEU2 allele, pWJ391 was cut at the unique SalI site at the 5' end of the coding sequence.
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<td>W933-15D</td>
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</table>
and at the XhoI site lying just beyond the termination codon before ligating in a 2.2-kb XhoI/SalI fragment bearing LEU2. This construction replaces the 3' end of SAM2 with LEU2.

In all cases, the in vitro disrupted genes were transformed into the yeast genome by single step gene disruption (ROTHSTEIN 1983). The presence of the mutant genes was detected both by the loss of SAM gene function which cosegregated with the dominant selectable marker in crosses, and by alterations in the structure of the SAM loci as detected by genomic blot analysis (SOUTHERN 1975; data not shown). Strains carrying deletion-disruption alleles at both loci are non-reverting AdoMet auxotrophs.

Frameshift mutations in the SAM genes were constructed by filling-in the BglII site at the 5' end of SAM1 and the SalI site at the 3' end of SAM2. These sites were chosen because they are at opposite ends of their respective genes and are within regions that differ greatly in their degree of homology with the corresponding regions of the other gene (Figures 1 and 2). These restriction sites were also chosen because filling them in creates a ClaI site in place of the BglII site, and a PvuI site in place of the SalI site. Therefore, the presence or absence of each mutation can be scored in a recombinant or revertant.

Plasmids containing each frameshift mutation and the URA3 gene were constructed (pWJ366 and pWJ367). Each plasmid was independently targeted to its homologous SAM locus by cutting within the sam gene before transformation (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). Mitotically stable Ura+ transformants contained direct repeats of the SAM locus with the wild-type and mutant SAM genes flanking URA3. The transformants were put onto medium containing 5-fluoro-orotic acid (5-FOA; BOEKE, LACROUZE and FINK 1984) to select for cells in which direct repeat recombination removed URA3 leaving only one copy of the SAM gene (SCHERER and DAVIS 1979). The 5-FOA-resistant yeast were then screened for AdoMet auxotrophy. DNA isolated from AdoMet auxotrophs was analyzed on genomic blots in order to detect the loss of the original restriction site and the acquisition of the new restriction site indicative of the mutant sam genes (data not shown). Cells containing a frameshift at one locus and a deletion at the other are sam auxotrophs confirming that the sam1 and sam2 frame-shift mutations lead to loss of gene function.

Reversion analysis: Single colonies from strains bearing a frame-shift mutation at one locus and a deletion-insertion at the other were used to inoculate each of 100 5 ml YPD + 0.1 mg/ml AdoMet cultures. The cells were grown to a density of 2–5 × 10^7 cells/ml at 30°, spun down and resuspended in 10 ml of YPD. Cultures incubated overnight at 30° were harvested and all of the cells from each tube were plated onto single YPD plates. The number of plates lacking AdoMet prototrophs after 5 days growth at 30° was used to determine the rate of prototrophy formation by the method of LURIA and DELBRUCK (1943) (Table 3). The reversion rate for each frame-shift mutant allele was determined by examining the reversion rates obtained in the sam1-ΔBglII SAM2::HIS3 strain and the sam1::LEU2 sam2::ΔSalI strain. The reversion rate in red52 strains bearing frameshift mutations at both SAM loci was also determined in this manner. To determine the nature of these reversion events, one colony from each plate containing prototrophs was subjected to genomic blot analysis as described below.

**Table 2**

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<td>SAM2</td>
<td>1.8-kb XhoI/HindIII SAM2 fragment in pGEM1</td>
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<tr>
<td>pWJ356</td>
<td>SAM1</td>
<td>1.7-kb XhoI/SnaBI SAM1 fragment in pGEM1</td>
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<td>pWJ357</td>
<td>sam2::HIS3</td>
<td>1.3-kb BamHI/XhoI HIS3 gene inserted into BglII/SalI cut SAM2 gene in pWJ355</td>
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<td>pWJ358</td>
<td>sam2-ΔSalI</td>
<td>Sam2 site in pWJ355 SAM2 gene filled-in creating PvuI site</td>
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<tr>
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<td>sam1::LEU2</td>
<td>2.0-kb BglI/SalI/LEU2 fragment inserted into BglII (filled-in)/SalI cut SAM1 gene in pWJ356</td>
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<td>BglII site in pWJ356 SAM1 filled-in creating ClaI site</td>
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<td>pWJ366</td>
<td>sam2-ΔSalI</td>
<td>1.2-kb HindIII URA3 fragment inserted into pWJ358. SmaI site in URA3 is oriented away from sam2-ΔSalI. Transcription units diverge</td>
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<td>SAM2</td>
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<td>2.2-kb XhoI/SalI LEU2 gene inserted into XhoI/SalI cut SAM2 gene in pWJ391</td>
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**Genomic DNA analysis of AdoMet prototrophs:** DNA was prepared from 10-ml saturated YPD cultures by the method of HOFFMAN and WINSTON (1987). The DNA was digested simultaneously with BglII and SalI restriction endonucleases (New England Biolabs) before electrophoresis and genomic blotting. Blots were simultaneously hybridized to radioactively labeled internal 460-bp EcoRV/SalI fragments of both the SAM1 and SAM2 genes (Figure 1A) and autoradiographed (Figure 2A). AdoMet prototrophs with BglII/SalI cleavage patterns that were indistinguishable from the starting auxotrophs were subjected to genetic analysis (as described below) to determine to which SAM locus the prototrophy maps. DNA isolated from prototrophs that...
FIGURE 1.—Maps and sequences of SAM1 and SAM2. (A) Protein coding sequences and distribution of tracts of extended homology. Relevant restriction sites as well as the translational start and stop sites and their locations within the SAM genes are depicted at the top of the figure. The linear scale at the top of the figure and the numbers in parentheses following the restriction sites represent base pairs from the ATG start codon. Note that the BglII and AhaII sites at the 5' end of SAM1 and the PvuI and NdeI sites at the 5' end of SAM2 are unique to those genes. A depiction of pairing between the mutant saml-ABglII and sam2-ASalI genes is at the bottom of the figure. The restriction sites used to mutate SAM1 (BglII) and SAM2 (SalI) are shown in bold type and with an A to designate that they are missing in the mutant genes. The method used to create the mutations creates new restriction sites (ABgII = ClaI, SamI = PvuI). Regions of uninterrupted identical sequence greater than 13 bp in length are represented by shaded boxes. The length of identical sequence is shown within each box. The boxes without numbers are 14 bp long. (B) Comparison of the DNA sequences of SAM1 and SAM2. The DNA sequence surrounding the BglII site in SAM1 and the SalI site in SAM2 are shown. The vertical hash marks denote sequence identity. The black circles denote mismatches between the two sequences. In addition to the SalI and BglII restriction sites used to make the mutations in saml-ABglII and sam2-ASalI, the polymorphic restriction sites PvuI and NdeI that flank the SalI site in SAM2, and the AhaII site that flanks the BglII site in SAM1 are denoted. These are used in determining conversion tract length.

map to SAM1 was digested simultaneously with ClaI and SalI, blotted, and probed with radioactively labeled 460-bp EcoRV/SalI fragment of the SAM1 gene (Figure 2B). DNA isolated from cells with prototroph mapping to SAM2 was digested with BglII and PvuI and probed with radioactively labeled 460-bp EcoRV/SalI fragment from the SAM2 gene (Figure 2C).

Prototroph segregation analysis: AdoMet prototrophs with BglII/SalI cleavage patterns that were indistinguishable from the starting, nonrecombinant strain were mated either to W744-1A or W744-2A, which bear deletion-disruption mutations at both SAM loci (saml::LeuZ, sam2::HIS3) and tetrads were dissected. If the AdoMet prototrophy was due to an event at the SAM1 locus then it always segregated in repulsion with the Leu+ prototrophy. If the AdoMet prototrophy was due to an event at the SAM2 locus then it always segregated in repulsion with the His+ prototrophy. All AdoMet prototrophs were linked to either one or the other of the SAM loci.

RESULTS

Homeologous conversion favors areas of greatest sequence homology: An assay was developed to examine recombination between the homeologous SAM
pared as described in MATERIALS AND METHODS and was digested
with BglII and SalI. The genotypes of the strains are listed across
the top of the lanes. The wild-type SAMI and SAM2 bands observed in the
original saml-AEgll gene results in a novel CAl site that have the original frame-shift mutation at SAM2 as denoted by
the absence of the SalI site in the coding sequence. (C) The nature of the recombination/reversion event was
determined by a genomic blot. DNA was prepared from a culture derived from a single AdoMet prototrophic colony (per plating) as
described in MATERIALS AND METHODS, digested with BglII and SalI
restriction endonucleases, blotted, and probed with nick translated internal 460-bp EcoRV/SalI fragments from SAM1 and SAM2 (see
Figure 1A). A representative autoradiogram is pictured here. The
genotypes of the strains are listed across the top of the lanes. The sizes of the bands in nucleotide base-pairs are denoted. Hybridization
reveals a 2.3-kb sam2-Sal1 band and a 1.1-kb saml-AEgll band in digests of DNA from the original Ado-Met auxotroph strain. The wild-type SAMI and SAM2 bands observed in the
protoporphs to determine conversion tract length and to identify
genes that are located on different chromosomes. Ectopic recombination assays were conducted using strains with frameshift mutations at both the SAM1 and SAM2 loci. These strains were grown to stationary phase (5.0 × 10^7 cells/ml) in non-selective liquid medium containing AdoMet and were then plated on AdoMet-free medium to determine the number of AdoMet prototrophs in the culture. The frequency of AdoMet prototroph formation was expressed as the number of prototrophs per viable cell plated. Rates of prototroph formation were determined using the median value from at least 37 independent trials (LEA and COULSON 1949), or by LURIA-DELBRUCK (1943) fluctuation analysis. The rate of AdoMet prototroph formation in wild-type cells was 8.4 × 10^-9 prototrophs/cell division (Table 3) and was more than 1,400-fold greater than the reversion rate in wild-type cells. This rate of prototroph formation is 8–23-fold lower than rates of prototroph formation in assays using duplicate homologous genes (JINKS-ROBERTSON and PETES 1986; LICHTEN and HABER 1989; A. BAILIS and R. ROTHSTEIN, unpublished observations).

Genomic blot analysis of DNA isolated from cultures of a single prototroph from each plating experiment was performed to determine the nature of the event leading to prototrophy (Figure 2, A–C). The predominant prototroph class in wild-type cells (92%) is due to a gene conversion event that restores the

binants have restored the SalI site in SAM2 but have retained the flanking PvuI and NdeI sites. The S-P class have restored the SalI site and converted away the PvuI site but not the NdeI site. S-P-N recombinants have restored the SalI site and converted away both the PvuI and NdeI sites. The size of each fragment is denoted in nucleotide base pairs. The sam2 bands in class O cells are 954 bp (BglII/PvuI) and 1109 bp (BglII/NdeI). The SAM2 bands in class S cells are 1024 bp (BglII/PvuI) and 1109 bp (BglII/NdeI). The SAM2 bands in class S-P cells are 2800 bp (BglII/PvuI) and 1109 bp (BglII/
NdeI). The SAM2 bands in S-P-N cells are 2800 bp in both digests. The sam1 bands are 5200 bp in both digests.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Rate (events/cell division)^a</th>
<th>Background^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WT</td>
<td>8.4 × 10^-9</td>
<td>0.006 × 10^-9</td>
</tr>
<tr>
<td></td>
<td>rad1</td>
<td>6.3 × 10^-9</td>
<td>0.008 × 10^-9</td>
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<tr>
<td></td>
<td>pms1</td>
<td>37.8 × 10^-10</td>
<td>0.020 × 10^-9</td>
</tr>
<tr>
<td></td>
<td>rad1 pms1</td>
<td>8.7 × 10^-9</td>
<td>0.018 × 10^-9</td>
</tr>
<tr>
<td>B</td>
<td>rad52</td>
<td>0.02 × 10^-8</td>
<td>0.040 × 10^-9</td>
</tr>
<tr>
<td></td>
<td>rad52 rad1</td>
<td>0.04 × 10^-8</td>
<td>ND^b</td>
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<tr>
<td></td>
<td>rad52 pms1</td>
<td>0.2 × 10^-8</td>
<td>0.150 × 10^-9</td>
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</table>

^a Group A (gene conversion) rates determined by the method of the median (LEA and COULSON 1949). Group B (reversion) rates determined by fluctuation analysis (LURIA and DELBRUCK 1943).

^b Background reversion rates are the sum of the rates determined for both sam genes by fluctuation analysis (LURIA and DELBRUCK 1943).

^c Not determined.
SAM2 gene to wild type and results in the restoration of the SalI site (Figure 3). The second type of AdoMet prototroph (5%) has a functional SAM1 gene that has lost the sam1-ΔBglII frameshift mutation as indicated by the loss of the ClaI site (ΔBglII—see Figures 1A and 3). These are likely due to gene conversion events that give rise to AdoMet prototrophs arising from strains containing sam1-ΔBglII and sam2-ΔSalI. The majority (63%) have lost the ClaI site indicative of the frameshift mutation in sam1-ΔBglII (data not shown). Thus, we conclude that the sam1-ΔBglII mutation can be restored using information from SAM2 to result in a functional SAM1 gene.

**Homeologous ectopic gene conversion is RAD52 dependent:** Cells bearing a lesion in the pleiotropic recombination and repair gene RAD52, are defective for mitotic recombination (PRAKASH et al. 1980; COLLINS et al. 1980; JACKSON and FINK 1981; WINSTON et al. 1984; ROHSTEIN, HELMS and ROSEMBERG 1987; KLEIN 1988a; THOMAS and ROHSTEIN 1990). The ectopic gene conversion events that give rise to AdoMet prototrophs are dependent on a wild-type RAD52 gene as there is no detectable recombination in rad52 mutants. The rate of AdoMet prototroph formation in rad52 mutant cells does not exceed the sum of the reversion rates of both sam1-ΔBglII and sam2-ΔSalI (Table 3), however, the reversion rate of each is 5–8-fold higher in rad52 cells than in wild type cells, due to the mutator phenotype of rad52 (HOEKSTRA, NAUGHTON and MALONE 1986; KUNZ et al. 1989). Genomic blot analysis of the AdoMet prototrophs arising in the rad52 background shows that the predominant event leading to prototrophy is one that leads to a restoration of the BglII site in sam1-ΔBglII (data not shown). This revertant is not due to a gene conversion event since there is no BglII site at the equivalent position in SAM2. In a rad52 background, the restoration of the BglII site occurs at the

### TABLE 4

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Rate (events/cell division)$^a$</th>
<th>Background$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sam1-ΔBglII, sam2-ΔSalI</td>
<td>8.4 × 10$^{-9}$</td>
<td>0.006 × 10$^{-9}$</td>
</tr>
<tr>
<td>sam1-ΔBglII, sam2::LEU2</td>
<td>0.3 × 10$^{-9}$</td>
<td>0.003 × 10$^{-9}$</td>
</tr>
</tbody>
</table>

$^a$ Gene conversion rate determined by the method of the median (LEA and COULSON 1949).

$^b$ Reversion rate determined by fluctuation analysis (Luria and Delbruck 1947).
same frequency even in the total absence of SAM2 sequences (data not shown). This unusual reversion event will be discussed further in a later section.

The distribution of coconversions is altered in rad1 mutants: We investigated whether mutations in the excision repair gene, RAD1, have an effect on mitotic recombination between the sam genes since recent results indicated that RAD1 and RAD52 occupy positions in two different pathways for mitotic recombination (KLEIN 1988a; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN, 1989). The rate of AdoMet prototroph formation in rad1 null mutant cells is not significantly different from the wild-type rate ($\chi^2 = 2.7, p = 0.11$; Table 3). Genomic blot analysis reveals a wild-type pattern with conversions of sam2-ΔSaiI to wild-type far outnumbering other conversion or reversion events (Figure 3). Additionally, AdoMet prototroph formation in rad1 rad52 double mutant cells occurs at the same low rate as in rad52 single mutants (Table 3). Thus, unlike direct repeat recombination no synergistic effect on recombination is observed.

An interesting difference was noted in the nature of the conversion events in rad1 and wild-type strains: namely their patterns of coconversion differed. The sam ectopic gene conversion assay permits coconversion to be scored using the PvuI and NdeI sites that are unique to SAM2 and lie downstream from the SalI site (see Figures 1, A and B, and 4A). The PvuI site is 67 bp and the NdeI site is 152 bp downstream from the SalI site. SAM2 convertants were analyzed by genomic blotting to determine if the event that restored the SalI site in the SAM2 gene resulted in the loss of the flanking PvuI and/or NdeI sites (Figure 4A). Conversion events that remove the flanking PvuI site, S-P coconversions, must be at least 68 bp long and conversions that remove both the PvuI and NdeI sites are disrupted. In wild-type cells approximately 25% of the conversions restoring the SalI site in SAM2 involve downstream sequences (Figure 4B). Of these conversions, 60% are S-P-N coconversions and 40% are S-P coconversions. No discontinuous coconversions involving SalI and NdeI but not PvuI were observed. In rad1 cells SAM2 conversion events involve downstream sequences 40% of the time and the distribution of these coconversions differs markedly from those in wild-type cells. The shorter S-P coconversions constitute 88% of those events and the S-P-N coconversions the remaining 12% (Figure 4B). This pattern of coconversion is significantly different from that of wild-type ($\chi^2 = 7.07, P = 0.03$).

Conversion tract analysis was also performed on all SAM1 prototrophs. In this case a single AhaI site...
flanking the BgIII site in SAM1 but absent in SAM2 can be used to determine whether a conversion event has occurred (Figure 1, A and B). None of the SAM1 prototrophs, regardless of the genetic background, was a coconversion (data not shown). Thus, the events leading to SAM1 restorations are due to conversions that do not extend far into adjacent homeologous sequences as well as reversions.

Hyper-recombination in a pmsl null mutant strain is dependent on RAD1: Heteroduplex DNA formation during recombination between homeologous genes could give rise to extensive amounts of mismatched DNA. For this reason we chose to look at recombination between the sam genes in a well-characterized mismatch repair defective strain. The pleiotropic mismatch repair and recombination mutation, pmsl, causes an increase in the frequency of postmeiotic and mitotic sectored colony formation (Williamson, Game and Fogel 1985; Bishop et al. 1987; Kramer et al. 1989a; J. McDonald and R. Rothstein, personal communication). The rate of mitotic heteroallelic recombination is also increased 3–5-fold in pmsl mutants (Williamson, Game and Fogel 1985). The rate of AdoMet prototroph formation in pmsl null mutant cells is 4.5-fold higher than in wild-type cells (Table 3). Like all recombination between the sam genes, pmsl-stimulated hyper-recombination is RAD52 dependent (Table 3). Genomic blot analysis of a representative number of prototroph colonies revealed that the pattern of conversion was identical to wild-type (Figure 3). In addition, a minor class of SAM1 prototrophs (2.5%) has restored the BgIII site and resembles the predominant class of revertants occurring in rad52 mutant cells. The rate of occurrence of these events in the pmsl rad52 double mutant is higher than the sum of the rates for each single mutant (Table 3).

Since RAD1 and several other excision repair pathway genes are required for the initiation of repair of many kinds of DNA damage including thymine dimers (Reynolds and Friedberg 1981; Wilcox and Prakash 1981) interstrand crosslinks (Miller, Prakash and Prakash 1982) and E. coli dam methylated DNA (Hoekstra, Naughton and Malone 1986), we reasoned that mismatched DNA sequences might result in helical distortions that are substrates for the excision repair machinery, and that rad1 mutations might change the effect of pmsl mutations on recombination. To test this hypothesis, we assayed ectopic gene conversion between the sam genes in a pmsl rad1 double mutant background. The presence of the rad1 mutation in the pmsl mutant background reduced the rate of ectopic gene conversion to a wild-type level (Table 3). Thus, the hyperrecombination phenotype in a pmsl mutant is dependent upon RAD1 function.

Coconversion was also analyzed in the pmsl rad1 double mutants to determine if loss of mismatch repair function leads to changes in the pattern of coconversion. The amount and distribution of coconversions in pmsl rad1 double mutants closely resembled those of rad1 cells (Figure 4B; χ² = 0.47, P = 0.79). Approximately a third of the conversion events were coconversions in pmsl rad1 cells with the S-P class of coconversions being 92% of the coconversions, and 8% of the coconversions being of the S-P-N class. In contrast, the distribution of coconversions in pmsl mutant cells was nearly identical to wild-type with 27% of the conversions involving the flanking restriction sites (Figure 4B; χ² = 0.03; P = 0.98). S-P class conversions comprised 37% of the coconversions while 63% of the coconversions were S-P-N coconversions. Therefore, PMS1 appears neither to be involved in determining the pattern of coconversion by itself, nor to be involved in establishing the coconversion phenotype of the rad1 mutant.

DISCUSSION

Following the duplication of single copy genes, random mutation causes the identical genes to rapidly diverge from each other since in most cases the function of only one of the two genes is acted upon by selection. As the gene pair diverges, each gene may become distinct either by changes in function, regulation, or both. Ectopic gene conversion acts as a force opposing divergence by homogenizing both genes (Nagylaki and Petes 1982). However, gene conversion requires substantial homology to occur at frequencies near the level of mutation ( Shen and Huang 1986; Watt et al. 1985; Waldman and Liskay 1987). Therefore, in order for gene conversion to continue to stabilize the homology between duplicate genes, extensive regions of homology must remain. It is the balance between the forces of mutation and conversion that simultaneously maintains the integrity and the diversity of multi-gene families.

We have used a naturally duplicated gene pair in Saccharomyces cerevisiae to examine the role of homology in ectopic recombination. In experiments with the homeologous SAM gene pair, we find that the rate of gene conversion is between 8- and 23-fold lower than the rates of ectopic conversion between dispersed copies of duplicate homologous sam1 (A. M. Bailis and R. Rothstein, unpublished data), leu2 (Lichten and Haber 1989), or ura3 (Jinks-Robertson and Petes 1986) genes. Lichten and Haber (1989) have shown that the rate of mitotic recombination between homologous genes at allelic locations is virtually identical to the rate observed when the genes are at ectopic sites. Taken together these results suggest that homology within the gene is an important determinant of the rate of ectopic recombination.

Genomic blot analysis of AdoMet prototrophs ob-
tained in recombination proficient strains revealed that the majority had restored one of the frameshift mutations to wild-type more frequently than the other. The bias in the frequency of conversion is likely due to the fact that one mutation (sam2-ΔSalI) lies within the largest span of continuous homology between the two genes (59 bp), while the other mutation (sam1-ΔBglII) lies within a region of relative nonhomology (Figure 1, A and B). Homology may be important during various stages of the conversion process. The initiation of pairing, formation of heteroduplex, as well as the extent of branch migration may all be dependent upon homology. The reduction in the rate of gene conversion observed between homoeologous genes could result from a change in any of these processes thereby reducing the probability that a particular mutant site is converted.

In addition to a change in the rate of conversion, a change in the incidence of coconversion might also be expected. Conversion tract length under conditions of limited homology can be estimated by the observation of coconversion of adjacent sites during recombination between the homeologous SAM genes. In wild-type cells, one-fourth of the conversions of sam2-ΔSalI coconvert one or both of a pair of 3' flanking restriction sites (Figure 4, A and B). In rad1 mutant cells, the frequency of coconversion is somewhat higher than wild-type, however, the distribution of events differs even more markedly (Figure 4B, χ² = 7.07, P = 0.03). In rad1 cells there are seven times more coconversions involving only the proximal restriction site (S-P) than coconversions involving both flanking sites (S-P-N; Figure 4B). In contrast, wild-type cells exhibit ½ fewer coconversions of the proximal site (S-P) than conversions of both flanking sites (S-P-N). No discontinuous conversions were observed, consistent with results observed in other laboratories (AHN and LIVINGSTON 1986; SYMINGTON and PETES 1988; BORTS and HABER 1989).

The coconversion results reveal that there are fewer of the longest class of coconversions and a greater number of the intermediate class of coconversions in rad1 cells than in wild-type cells. A similar reduction in the amount of long coconversions has been observed during recombination between homologous inverted repeats in rad1 cells (AGUILERA and KLEIN 1989). The rate of conversion in either system is not affected significantly by the change in the distribution of coconversions. Since only one end of the conversion tract is being examined in our experiments, we can not rule out the possibility that rad1 does not change tract length but instead changes where recombination between the mismatched sam genes tends to initiate or terminate. For example, initiation of conversion either farther 3' or 5' from where recombination is normally initiated, and the maintenance of wild-type conversion tract length, would change the frequency of conversion of the flanking restriction sites since they would be included in the conversion tract with altered frequency. This explanation is unlikely since there is very little continuous homology 5' to the SalI site (see Figure 1, A and B) with which to initiate conversion. Experiments by Liskay and coworkers support this viewpoint (WALDMAN and LISKAY 1988). They have shown that conversion in mammalian cells can involve extensively mismatched (19%) DNA but that these events require significant stretches of identical sequence in which to initiate. Although it is as yet unclear how the loss of excision repair function in rad1 cells might change conversion tract length, one possibility may be that RAD1 affects the mobility of HOLLIDAY (1964) junctions. Alternatively, RAD1 may determine the likelihood of HOLLIDAY junction resolution in a particular region.

Wild-type function of the double-strand-break repair gene, RAD52, is necessary for mitotic recombination between the sam genes. The rate of mitotic ectopic gene conversion in rad52 cells is undetectable being below the rate of reversion (Table 3). The rad52 mutation is epistatic to both the excision repair mutation rad1 and the mismatch repair mutation pms1 (Table 3). The absolute dependence of mitotic ectopic gene conversion on RAD52 suggests that RAD52 controls the sole pathway for this type of recombination event. The failure to observe discontinuous conversion tracts in this assay coupled with the complete dependence on RAD52 argues that these recombaints arise by double-strand-break repair.

In addition to its role in recombination, RAD52 plays a role in mutation (PRAKASH et al. 1980). Defects in RAD52 function lead to a 7-fold increase in the reversion rate of both mutant sam genes (Table 3). The predominant AdoMet prototroph arising in rad52 cultures is due to an event that restores the BglII site to sam1-ΔBglII. This event can not be due to gene conversion as there is no BglII site in SAM2 with which to convert the frameshift mutation in sam1-ΔBglII to wild type. This event also occurs in strains in which SAM2 sequences are completely absent. Slippage and mispairing during replication of the palindromic sequences that surround the mutant site may account for this aberrant event (EFSTRA-TIADIS et al. 1980). Mutations in the PMS1 gene also lead to an increase in the rate of recovery of this aberrant type of prototroph (Table 3). One possible explanation for this observation is that at some low frequency wild-type cells form an intermediate that is normally removed by either RAD52 or PMS1. In the absence of their function, processing of this intermediate leads to a deletion of 4 bases and the restoration of the BglII site.

The rad52 and pms1 mutations together exert a
synergistic effect on the reversion rate (Table 3). On the other hand, the 4.5-fold increase of recombination observed in \textit{pmsl} mutants occurs via a \textit{RAD52} dependent pathway (Table 3). This increased level is similar to the increase in heteroallelic recombination seen at a number of loci in \textit{pmsl} mutant strains (3–5-fold; Williamson, Game and Fogel 1985). In addition, recent experiments in our laboratory have shown that the \textit{pmsl} mutation leads to the same increase in the rate of ectopic recombination between homologous \textit{saml} mutant genes (4.5-fold) as that reported here for recombination between the homeologous \textit{saml} and \textit{sam2} genes (Table 3; A. M. Bailis and R. Rothstein, unpublished observations). The increased recombination can not be due solely to the preservation of heteroduplex DNA since replication of unrepaired heteroduplex would increase the number of recombinants a maximum of 2-fold.

It is interesting that the \textit{pmsl}-dependent increase in recombination between homeologous genes is similar to the increase observed for homologous genes. Recently Radman and his colleagues have postulated that the mismatch repair machinery prevents exchange between dissimilar sequences by aborting heteroduplexes that are formed between them (Radman 1988; Rayssiguier, Thaler and Radman 1989). If this were true for our assay, one might expect \textit{pmsl} mutant cells to exhibit a much larger increase in the rate of recombination between the extensively mismatched \textit{sam} genes than that seen between homologous heteroalleles. An alternative explanation is suggested by the increased spontaneous mutation rate observed in \textit{pmsl} cells. Perhaps wild-type \textit{PMS1} function is important in processing mismatches that occur as the result of the mis-incorporation of nucleotides during DNA replication. The mismatches that accumulate in the absence of \textit{PMS1} may lead to an increase in recombinogenic lesions. This would lead to an equivalent effect on recombination between homologous and homeologous genes.

The hyper-recombination seen in the \textit{pmsl} mutant strains is dependent upon the excision repair pathway gene, \textit{RAD1} (Table 3). Wild-type \textit{RAD1} is involved in the incision step of the excision repair of thymine dimers (Reynolds and Friedberg 1981; Wilcox and Prakash 1981), interstrand crosslinks (Miller, Prakash and Prakash, 1982) and of \textit{dam} methylated DNA (Hoekestra, Naughton and Malone 1986). We suggest that \textit{RAD1} may also recognize the mismatches accumulating in \textit{pmsl} mutant cells. \textit{RAD1} may act by incising adjacent to the mismatches marking them for uptake into the \textit{RAD52} recombination pathway (Figure 5). We believe that \textit{RAD1} acts after the point where the \textit{pmsl} mutation is affecting DNA metabolism because \textit{rad1} mutations do not block the hyper-mutation phenotype of \textit{pmsl} in the double muta-

**Figure 5.**—A model explaining the hyperrecombination phenotype in mismatch repair defective \textit{pmsl} mutant cells. (A) A prototypical gene is depicted as double stranded DNA prior to DNA replication. (B) DNA replication occurs and mismatches accumulate due to a defect in mismatch repair imposed by the \textit{pmsl} mutation. (C) The excision repair machinery, of which \textit{RAD1} is part, recognizes the mismatch and cleaves on either side leaving a single-strand gap. (D) Single-strand gapped DNA enters directly into the \textit{RAD52}-dependent recombination pathway or is processed into a double-strand break before entering (not shown).

A similar model has been proposed to account for the epistatic interactions between the mitotic hyper-rec mutation \textit{rad3-101}, \textit{rad1} and \textit{rad52} (Montelone, Hoekestra and Malone 1988). In that model the \textit{rad3-101} mutation leads to an increase in the number of mismatches due to misreplication. These mismatches are then processed by a \textit{RAD1}-dependent step into recombinogenic lesions that are thought to be double-strand-breaks since they require \textit{RAD52} (or \textit{RAD50}) to be processed. Physical evidence for the accumulation of double-strand breaks in a \textit{rad3-101} background supports this notion (Montelone, Hoekestra and Malone 1988). While \textit{rad3-101 rad52} double mutants are inviable, \textit{pmsl rad52} double mutants resemble \textit{rad52} single mutants (data not shown). Since we do not see any lethality associated with the \textit{pmsl rad52} double mutant, we suggest that the \textit{pmsl} mutation does not lead to the accumulation of a lethal level of double-strand breaks.
The homeologous ectopic recombination assay introduced in this study has allowed us to investigate the interrelationship between recombination and DNA repair in yeast. Our genetic analysis suggests that the rate of mitotic recombination between the sam genes is dependent upon intimate communication between the processes of mismatch repair, excision repair and recombinational repair.

We are grateful to Jean-Luc Rossignol for discussions that led to the development of this assay. We thank Andreas Aguilera for his critical evaluation of the manuscript. We also thank Hannah Klein, Lorraine Symington and Miklos Radman for their comments on the manuscript. Thanks are due to Wilfried and Barbara Thomas and Hans Ronne for their help. A.M.B. was supported by National Institutes of Health (NIH) postdoctoral fellowship GM 34587 and a grant from the Irma T. Hirsch Trust.

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