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 8.4×10^{-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 required to observe this increased rate of ectopic conversion. In addition, *RAD1* 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 gene 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; POTIER, WINSOR and LACROUTE, 1982; SUGAWARA and SZOSTAK 1983; FASULLO and DAVIS 1987) and *Schizosaccharomyces pombe* (SZANKASI *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 ∂ elements (WINSTON *et al.* 1984; ROTHSTEIN, HELMS and ROSENBERG 1987), we well 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 93% 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 PETES 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 homeologous 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 coconversion 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 *hexB* 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 tetrad 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 tetrad 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 *Xho*I/*Hin*dIII DNA fragment bearing the *SAM2* gene into the *Sal*I and *Hin*dIII sites in the polylinker of pGEM1 (Promega Biotec). pWJ391 was constructed by cloning a 2.5-kb *Eco*RI/*Hin*dIII fragment containing the *SAM2* gene with additional 3′ flanking DNA into pGEM2, replacing the polylinker. pWJ356 was constructed by cloning a 1.7-kb *Xho*I/*Sna*BI fragment containing the *SAM1* gene into the *Sal*I and *Sma*I 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 *Bgl*II within the *SAM1* coding sequence (Figure 1A), flushed with Klenow (Boehringer-Mannheinm), cut at the unique *Sal*I site and then ligated to a 2.0-kb *Hpa*I/*Sal*I *LEU2* fragment. This results 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 *Bgl*II, which lies 5′ to the translational start codon of *SAM2*, and at the unique *Sal*I site within the *SAM2* coding sequence before ligating in the 1.3-kb *Bam*HI/*Xho*I *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 *Sal*I site at the 3′ end of the coding sequence

TABLE 1

Strains

Strain	Genotype	Origin
W303-1A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1*	ROTHSTEIN laboratory
W303-1B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1*	ROTHSTEIN laboratory
J491	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3*	This study
J492	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI*	This study
W675-1D	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad1::LEU2, rad52::TRP1, GAL10::URA3*	B. THOMAS
W744-1A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2::HIS3*	This study
W744-2A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2::HIS3*	This study
W763-2B	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI*	This study
W804-2D	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad1::LEU2*	This study
W804-3B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad1::LEU2*	This study
W804-2A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad52::TRP1*	This study
W804-9B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad52::TRP1*	This study
W804-7D	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad1::LEU2, rad52::TRP1*	This study
W804-8B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, rad1::LEU2, rad52::TRP1*	This study
W829-7D	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, rad1::LEU2*	This study
W829-22D	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, rad1::LEU2*	This study
W829-1C	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, rad52::TRP1*	This study
W830-7D	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, rad1::LEU2*	This study
W830-8A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, rad1::LEU2*	This study
W830-36A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, rad52::TRP1*	This study
W830-37B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, rad52::TRP1*	This study
W837-2B	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::LEU2*	This study
W837-9A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::LEU2*	This study
W840-4A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, pms1::LEU2*	J. MCDONALD
W848-5B	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, pms1::LEU2*	This study
W848-8C	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, pms1::LEU2*	This study
W869-3C	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, pms1::LEU2*	This study
W869-13B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, pms1::LEU2*	This study
W870-2A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, pms1::LEU2*	This study
W880-7D	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, pms1::LEU2, rad1::LEU2*	This study
W885-9A	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2-ΔSalI, pms1::LEU2,rad52::TRP1*	This study
W887-1C	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, pms1::LEU2,rad52::TRP1*	This study
W887-4A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1::LEU2, sam2-ΔSalI, pms1::LEU2, rad52::TRP1*	This study
W892-1D	*MAT***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, pms1::LEU2, rad52::TRP1*	This study
W892-2B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, pms1::LEU2, rad52::TRP1*	This study
W933-15D	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, pms1::LEU2, rad1::LEU2*	This study
W933-16A	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, sam1-ΔBglII, sam2::HIS3, pms1::LEU2, rad1::LEU2*	This study

TABLE 2

Plasmids

Plasmid	Relevant gene	Description
pWJ355	*SAM2*	1.8-kb *Xho*I/*Hin*dIII *SAM2* fragment in pGEM1
pWJ356	*SAM1*	1.7-kb *Xho*I/*Sna*BI *SAM1* fragment in pGEM1
pWJ357	*sam2::HIS3*	1.3-kb *Bam*HI/*Xho*I *HIS3* gene inserted into *Bgl*II/*Sal*I cut *SAM2* gene in pWJ355
pWJ358	*sam2-ΔSal*I	*Sal*I site in pWJ355 *SAM2* gene filled-in creating *Pvu*I site
pWJ359	*sam1::LEU2*	2.0-kb *Hpa*I/*Sal*I *LEU2* fragment inserted into *Bst*EII (filled-in)/*Sal*I cut *SAM1* gene in pWJ356
pWJ360	*sam1-ΔBgl*II	*Bgl*II site in pWJ356 *SAM1* filled-in creating *Cla*I site
pWJ366	*sam2-ΔSal*I	1.2-kb *Hin*dIII *URA3* fragment inserted into pWJ358. *Sma*I site in *URA3* is oriented away from *sam2-ΔSal*I. Transcription units diverge
pWJ367	*sam1-ΔBgl*II	1.2-kb *Hin*dIII *URA3* fragment inserted into pWJ360. *Sma*I site in *URA3* is oriented toward *sam1-ΔBgl*II. Transcription units converge
pWJ391	*SAM2*	2.5-kb *Eco*RI/*Hin*dIII *SAM2* fragment inserted into pGEM2
pWJ392	*sam2::LEU2*	2.2-kb *Xho*I/*Sal*I *LEU2* gene inserted into *Xho*I/*Sal*I cut *SAM2* gene in pWJ391

and at the *Xho*I site lying just beyond the termination codon before ligating in a 2.2-kb *Xho*I/*Sal*I fragment bearing *LEU2*. This construction replaces the 3′ end of *SAM2* with *LEU2*.

In all cases, the *in vitro* disrupted genes were transplaced 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 *Bgl*II site at the 5′ end of *SAM1* and the *Sal*I 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 *Cla*I site in place of the *Bgl*II site, and a *Pvu*I site in place of the *Sal*I 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, LACROUTE 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 prototroph 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-ΔBgl*II *SAM2::HIS3* strain and the *SAM1::LEU2 sam2-ΔSal*I strain. The reversion rate in *rad52* 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.

Homeologous ectopic recombination assay: For most genetic backgrounds, 10-ml YPD cultures supplemented with 0.1 mg/ml AdoMet were inoculated with single colonies of AdoMet auxotrophic mutants bearing frame-shift mutations at both *SAM* loci. The cultures were grown to a density of 5.0 × 10^7 cells/ml at 30°. Following growth, appropriate dilutions of these cultures were plated on YPD + AdoMet to determine the number of viable cells in the culture. The remaining cells were spun down, resuspended in 1 ml of H_2O, and 0.1-ml aliquots were plated on individual YPD plates to determine the rate of formation of AdoMet prototrophs. After 3 days of incubation at 30°, the total number of AdoMet prototrophs on the 10 plates was counted and the frequency of AdoMet prototroph formation determined. The median frequency value from among a total of at least 35 experiments was used to determine the rate by the method of LEA and COULSON (1949). To determine the nature of the event leading to prototrophy, DNA isolated from a culture grown from a single colony from each plating was analyzed by genomic blot analysis.

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 *Bgl*II and *Sal*I restriction endonucleases (New England Biolabs) before electrophoresis and genomic blotting. Blots were simultaneously hybridized to radioactively labeled internal 460-bp *Eco*RV/*Sal*I fragments of both the *SAM1* and *SAM2* genes (Figure 1A) and autoradiographed (Figure 2A). AdoMet prototrophs with *Bgl*II/*Sal*I cleavage patterns that were indistinguishable from the starting auxotroph were subjected to genetic analysis (as described below) to determine to which *SAM* locus the prototrophy maps. DNA isolated from prototrophs that

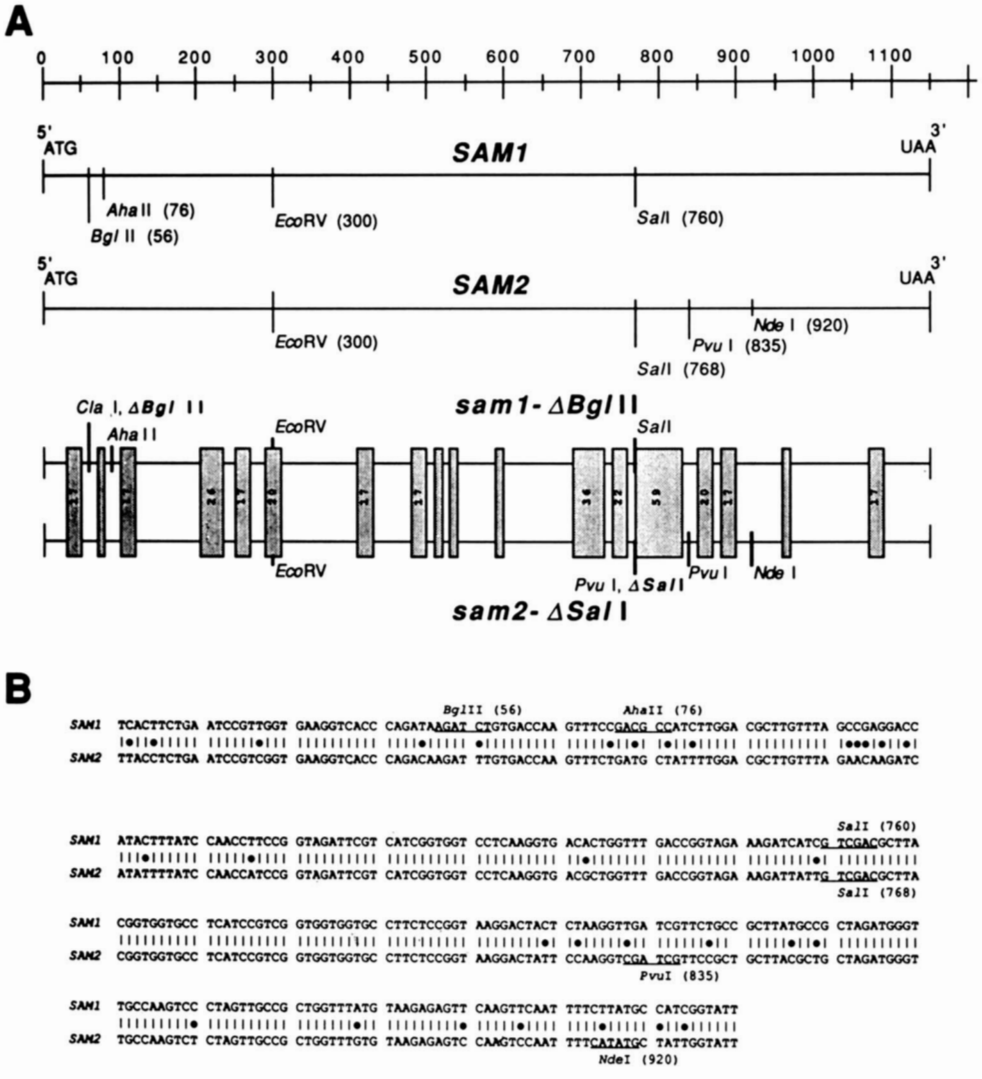

FIGURE 1.—Maps and sequences of *SAM1* and *SAM2*. (A) Protein coding sequences and distribution of tracts of extended homology. Relevant restriction sites as well as the translational start and stop sites and their locations within the *SAM* genes are depicted at the top of the figure. The linear scale 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 *Bgl*II and *Aha*II sites at the 5′ end of *SAM1* and the *Pvu*I and *Nde*I sites at the 5′ end of *SAM2* are unique to those genes. A depiction of pairing between the mutant *sam1*-Δ*Bgl*II and *sam2*-Δ*Sal*I genes is at the bottom of the figure. The restriction sites used to mutate *SAM1* (*Bgl*II) and *SAM2* (*Sal*I) are shown in bold type and with a Δ to designate that they are missing in the mutant genes. The method used to create the mutations creates new restriction sites (Δ*Bgl*II = *Cla*I, Δ*Sal*I = *Pvu*I). 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 *Bgl*II site in *SAM1* and the *Sal*I 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 *Sal*I and *Bgl*II restriction sites used to make the mutations in *sam1*-Δ*Bgl*II and *sam2*-Δ*Sal*I, the polymorphic restriction sites *Pvu*I and *Nde*I that flank the *Sal*I site in *SAM2*, and the *Aha*II site that flanks the *Bgl*II site in *SAM1* are denoted. These are used in determining conversion tract length.

map to *SAM1* was digested simultaneously with *Cla*I and *Sal*I, blotted, and probed with radioactively labeled 460-bp *Eco*RV/*Sal*I fragment of the *SAM1* gene (Figure 2B). DNA isolated from cells with prototrophy mapping to *SAM2* was digested with *Bgl*II and *Pvu*I and probed with radioactively labeled 460-bp *Eco*RV/*Sal*I fragment from the *SAM2* gene (Figure 2C).

Prototroph segregation analysis: AdoMet prototrophs with *Bgl*II/*Sal*I 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 (*sam1::Leu2*, *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 prototrophies 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*

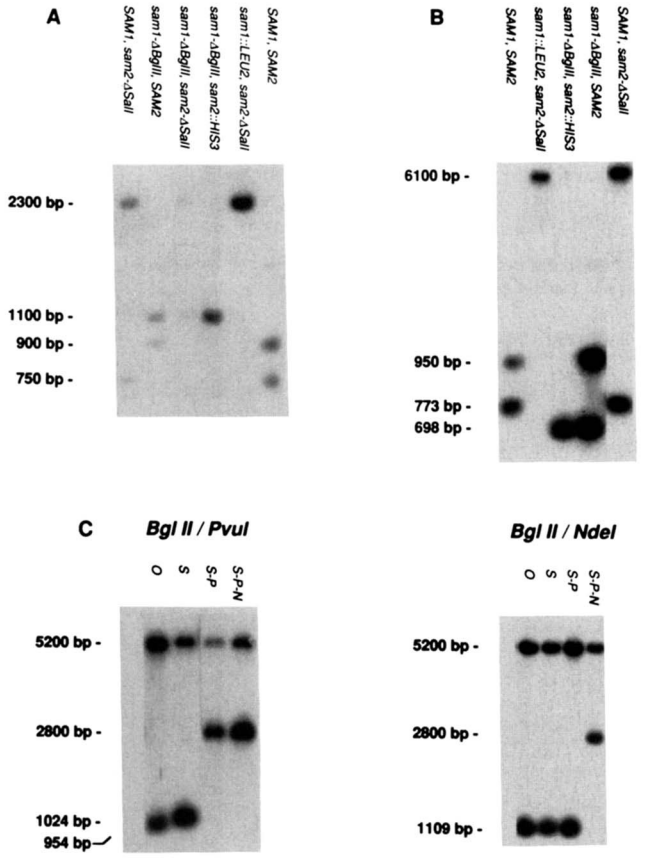

FIGURE 2.—Genomic blot of DNA isolated from AdoMet prototrophs. (A) The nature of the recombination/reversion event was determined by a genomic blot. DNA was prepared from a culture derived from a single AdoMet prototrophic colony (per plating) as described in MATERIALS AND METHODS, digested with *Bgl*II and *Sal*I restriction endonucleases, blotted, and probed with nick translated internal 460-bp *Eco*RV/*Sal*I 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*-Δ*Sal*I band and a 1.1-kb *sam1*-Δ*Bgl*II band in digests of DNA from the original Ado-Met auxotroph strain. The wild-type *SAM1* and *SAM2* bands observed in the prototrophs are 700 and 950 bp, respectively. (B) DNA isolated from strains in which prototrophy maps to the *SAM1* locus was subjected to genomic blot analysis. DNA was prepared as described in MATERIALS AND METHODS, digested with *Cla*I and *Sal*I restriction endonucleases and hybridized with the 460-bp *Eco*RV/*Sal*I fragment from *SAM1*. 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. The mutation in the original *samI*-Δ*Bgl*I gene results in a novel *Cla*I site and yields a 700-bp *sam1* band. In the *SAM1* prototrophs in which the frameshift mutation is lost but the *Bgl*II site is not restored a 773-bp *SAM1* band is observed. The wild-type *SAM2* gene yields a 950-bp band while the *sam2*-Δ*Sal*I mutant gene gives a 6100-bp band due to the absence of the *Sal*I site in the coding sequence. (C) Genomic blot analysis was performed on DNA isolated from *SAM2* prototrophs to determine conversion tract length and to identify reversion events. DNA from all *SAM2* prototroph strains was prepared as described in MATERIALS AND METHODS and was digested with *Bgl*II and *Pvu*I or *Bgl*II and *Nde*I. The conversion class is listedacross the top of each lane. The *O* class represents prototrophs that have the original frame-shift mutation at *SAM2* as denoted by the retention of the Δ*Sal*I sequence (*Pvu*I site, see Figure 1). *SAM2* prototrophs carrying this gene are revertants. The *S* class of recombinants have restored the *Sal*I site in *SAM2* but have retained the flanking *Pvu*I and *Nde*I sites. The *S-P* class have restored the *Sal*I site and converted away the *Pvu*I site but not the *Nde*I site. *S-P-N* recombinants have restored the *Sal*I site and converted away both the *Pvu*I and *Nde*I sites. The size of each fragment is denoted in nucleotide base pairs. The *sam2* bands in class *O* cells are 954 bp (*Bgl*II/*Pvu*I) and 1109 bp (*Bgl*II/*Nde*I). The *SAM2* bands in class *S* cells are 1024 bp (*Bgl*II/*Pvu*I) and 1109 bp (*Bgl*II/*Nde*I). The *SAM2* bands in class *S-P* cells are 2800 bp (*Bgl*II/*Pvu*I) and 1109 bp (*Bgl*II/*Nde*I). The *SAM2* bands in *S-P-N* cells are 2800 bp in both digests. The *sam1* bands are 5200 bp in both digests.

TABLE 3

Rates of AdoMet prototroph formation in wild-type (WT) and mutant strains

Group	Genotype	Rate (events/cell division)[a]	Background[b]
A	WT	8.4×10^{-9}	0.006×10^{-9}
	rad1	6.3×10^{-9}	0.008×10^{-9}
	pms1	37.8×10^{-9}	0.020×10^{-9}
	rad1 pms1	8.7×10^{-9}	0.018×10^{-9}
B	*rad52*	0.02×10^{-9}	0.040×10^{-9}
	rad52 rad1	0.04×10^{-9}	ND[c]
	rad52 pms1	0.2×10^{-9}	0.130×10^{-9}

[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.

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

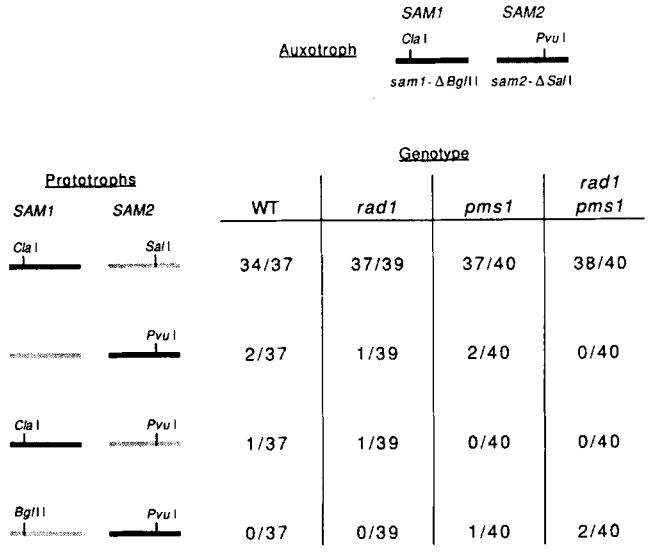

FIGURE 3.—AdoMet prototroph DNA patterns. DNA from a single AdoMet prototrophic colony from each culture plated was prepared and analyzed by genomic blotting as described previously. The results for all of the experiments involving recombination proficient strains are depicted here. Linkage of the AdoMet prototrophy to either the *SAM1* or the *SAM2* locus in strains that are indistinguishable from the starting auxotrophs on genomic blots was accomplished as described in MATERIALS AND METHODS. The gene conferring AdoMet prototrophy is represented by a lightly shaded line. The dark lines represent mutant alleles.

SAM2 gene to wild type and results in the restoration of the *Sal*I site (Figure 3). The second type of AdoMet prototroph (5%) has a functional *SAM1* gene that has lost the *sam1-ΔBgl*II frameshift mutation as indicated by the loss of the *Cla*I site (ΔBglII—see Figures 1A and 3). These are likely due to gene conversion events that repair the *sam1-ΔBgl*II mutation with information from *SAM2*. As *SAM2* does not have a *Bgl*II site at the same position as the *Bgl*II site mutated in *sam1-ΔBgl*II, information exchange in this region can not restore the *Bgl*II site in *sam1*. Alternatively these prototrophs are due to the restoration of the reading frame by a compensatory frame-shift mutation within the *Cla*I site. In a third class (3%), the prototrophy maps to *SAM2* but genomic blots show that neither the *Sal*I site has been restored, nor the *Pvu*I (*ΔSal*I—see Figure 1A) site lost (Figure 3). These prototrophs likely arise by rare compensatory frameshift mutation events that restore the reading frame and lie outside of the *Pvu*I site.

The results discussed above reveal a bias in the efficiency of conversion of the *sam1-ΔBgl*II and *sam2-ΔSal*I mutant genes. The *Sal*I site used to construct the *sam2* frameshift mutation is located next to the largest span of uninterrupted homology between *SAM1* and *SAM2* (59 bp) while the *Bgl*II site in *SAM1* is within an area of mismatch (Figures 1 and 2). The recombination results suggest that homology is an important determinant of where ectopic gene conversion occurs between homeologous genes. However,

TABLE 4

Rates of AdoMet prototroph formation in strains with full-length and 3′-end deleted *sam2* genes

Relevant genotype	Rate (events/cell division)[a]	Background[b]
*sam1-ΔBgl*II, *sam2-ΔSal*I	8.4×10^{-9}	0.006×10^{-9}
*sam1-ΔBgl*II, *sam2::LEU2*	0.3×10^{-9}	0.003×10^{-9}

[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).

an alternative explanation for the bias in conversion of the two *sam* genes is that a recombinant *SAM1* gene with its *Bgl*II region donated by *SAM2* does not encode a functional AdoMet synthetase. This possibility has been addressed by looking at recombination in a strain that contains the *sam1-ΔBgl*II allele and a *sam2* gene that deletes the *Sal*I site and all of the sequence 3′ to it. All AdoMet prototrophs arising from this strain are, therefore, either conversions of *sam1-ΔBgl*II to *SAM1*, or reversions. AdoMet prototrophs arise at a similar rate in this strain (3.0×10^{-10}, Table 4) as *SAM1* prototrophs arise in the strain containing *sam1-ΔBgl*II and *sam2-ΔSal*I (4.5×10^{-10}). This rate is 100-fold higher than the reversion rate of the *sam1-ΔBgl*II allele (Table 4). The majority (63%) have *SAM1* function restored and have lost the *Cla*I site indicative of the frameshift mutation in *sam1-ΔBgl*II (data not shown). Thus, we conclude that the *sam1-ΔBgl*II mutation can be converted 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; MALONE and ESPOSITO 1980; JACKSON and FINK 1981; WINSTON *et al.* 1984; ROTHSTEIN, HELMS and ROSENBERG 1987; KLEIN 1988a; THOMAS and ROTHSTEIN 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-ΔBgl*II and *sam2-ΔSal*I (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 *Bgl*II site in *sam1-ΔBgl*II (data not shown). This revertant is not due to a gene conversion event since there is no *Bgl*II site at the equivalent position in *SAM2*. In a *rad52* background, the restoration of the *Bgl*II site occurs at the

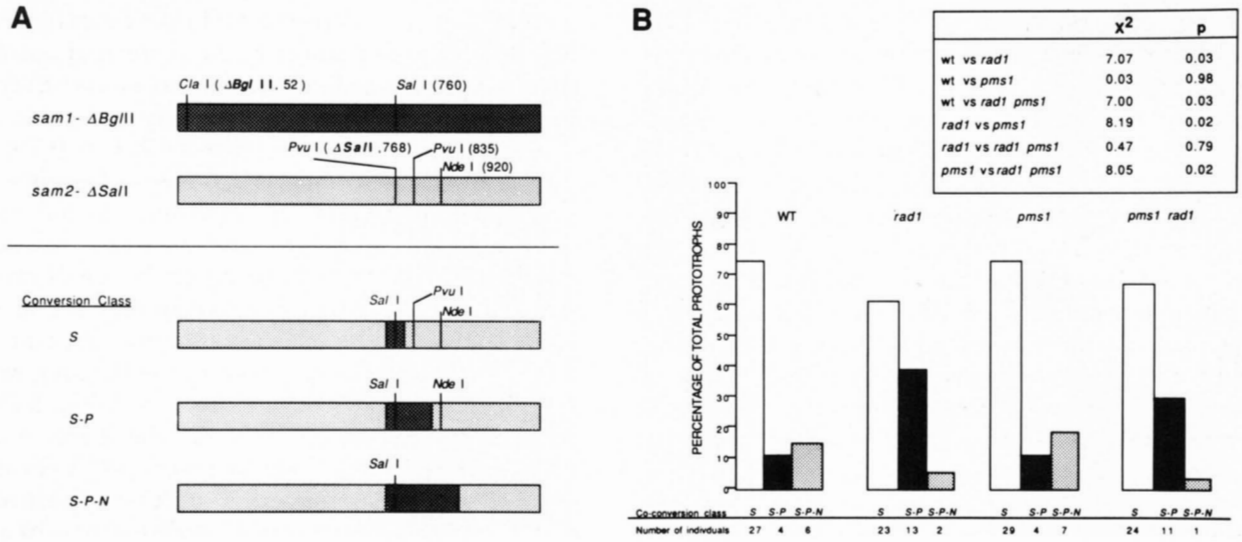

FIGURE 4.—Tract length and distribution after homeologous gene conversion. (A) DNA was isolated from cultures of the AdoMet prototrophs that contain recombinant *SAM2* genes, and was digested with *Bgl*II and *Pvu*I or *Bgl*II and *Nde*I, blotted and hybridized as described in the text. The parental mutant *sam* genes are pictured at the top of the diagram. *sam1-ΔBgl*II sequence is represented by the darkly shaded bar. *sam2-ΔSal*I sequence is represented by the lightly shaded bar. The pertinent restriction sites are shown with their positions relative to the transcriptional start codon shown in parentheses. The recombinant *SAM2* genes are shown at the bottom of the diagram. Recombinants in which only the *Sal*I site is restored are *S* conversions and are depicted with donated *sam1-ΔBgl*II sequence surrounding only the *Sal*I site. *S-P* coconversions are missing the *Pvu*I site and are depicted with *sam1-ΔBgl*II sequence extending past the *Pvu*I position. *S-P-N* coconversions are missing both the *Pvu*I and *Nde*I site and are depicted with *sam1-ΔBgl*II sequence extending past the *Nde*I position. (B) Conversion tract length distribution in wild-type and mutant backgrounds was determined by analyzing all of the recombinant *SAM2* genes resulting from gene conversion in recombination proficient strains as described in Figure 2C. The number of recombinants in each coconversion class (*S*, *S-P*, *S-P-N*) was determined and the number expressed as the percentage of the total number of prototrophs of that genetic background that were analyzed. The insert shows the results of a 2 × 3 contingency χ^2 analysis of the data.

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 (χ^2 = 2.7, p = 0.11; Table 3). Genomic blot analysis reveals a wild-type pattern with conversions of *sam2-ΔSal*I 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 *Pvu*I and *Nde*I sites that are unique to *SAM2* and lie downstream from the *Sal*I site (see Figures 1, A and B, and 4A). The *Pvu*I site is 67 bp and the *Nde*I site is 152 bp downstream from the *Sal*I site. *SAM2* convertants were analyzed by genomic blotting to determine if the event that restored the *Sal*I site in the *SAM2* gene resulted in the loss of the flanking *Pvu*I and/or *Nde*I sites (Figure 4A). Conversion events that remove the flanking *Pvu*I site, *S-P* coconversions, must be at least 68 bp long and conversions that remove both the *Pvu*I and *Nde*I sites, *S-P-N* coconversions, must be at least 153 bp long.

In wild-type cells approximately 25% of the conversions restoring the *Sal*I 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 *Sal*I and *Nde*I but not *Pvu*I 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 *Aha*II site

flanking the *Bgl*II 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 *pms1* 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, *pms1*, 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 *pms1* mutants (WILLIAMSON, GAME and FOGEL 1985). The rate of AdoMet prototroph formation in *pms1* null mutant cells is 4.5-fold higher than in wild-type cells (Table 3). Like all recombination between the *sam* genes, *pms1*-stimulated hyperrecombination 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 *Bgl*II site and resembles the predominant class of revertants occurring in *rad52* mutant cells. The rate of occurrence of these events in the *pms1 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 *pms1* mutations on recombination. To test this hypothesis, we assayed ectopic gene conversion between the *sam* genes in a *pms1 rad1* double mutant background. The presence of the *rad1* mutation in the *pms1* mutant background reduced the rate of ectopic gene conversion to a wild-type level (Table 3). Thus, the hyperrecombination phenotype in a *pms1* mutant is dependent upon *RAD1* function.

Coconversion was also analyzed in the *pms1 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 *pms1 rad1* double mutants closely resembled those of *rad1* cells (Figure 4B; $\chi^2 = 0.47$, $P = 0.79$). Approximately a third of the conversion events were coconversions in *pms1 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 *pms1* mutant cells was nearly identical to wild-type with 27% of the conversions involving the flanking restriction sites (Figure 4B; $\chi^2 = 0.03$; $P = 0.98$). *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 homeologous 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, $\chi^2 = 7.07$, $P = 0.03$). 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 ⅓ 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 LIVINGSTON1986; 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 recombinants 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 (EFSTRATIADIS *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 *pms1* mutants occurs via a *RAD52* dependent pathway (Table 3). This increased level is similar to the increase in heteroallelic recombination seen at a number of loci in *pms1* mutant strains (3–5-fold; WILLIAMSON, GAME and FOGEL 1985). In addition, recent experiments in our laboratory have shown that the *pms1* mutation leads to the same increase in the rate of ectopic recombination between homologous *sam1* mutant genes (4.5-fold) as that reported here for recombination between the homeologous *sam1* and *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 *pms1*-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 *pms1* mutant cells to exhibit a much larger increase in the rate of recombination between the extensively mismatched *sam* genes than that seen between homologous heteroalleles. An alternative explanation is suggested by the increased spontaneous mutation rate observed in *pms1* cells. Perhaps wild-type *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 *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 *pms1* mutant strains is dependent upon the excision repair pathway gene, *RAD1* (Table 3). Wild-type *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 *dam* methylated DNA (HOEKSTRA, NAUGHTON and MALONE 1986). We suggest that *RAD1* may also recognize the mismatches accumulating in *pms1* mutant cells. *RAD1* may act by incising adjacent to the mismatches marking them for uptake into the *RAD52* recombination pathway (Figure 5). We believe that *RAD1* acts after the point where the *pms1* mutation is affecting DNA metabolism because *rad1* mutations do not block the hyper-mutation phenotype of *pms1* in the double mutant (Table 3). A similar model has been proposed to account for the epistatic interactions between the mitotic hyper-rec mutation *rad3-101*, *rad1* and *rad52* (MONTELONE, HOEKSTRA and MALONE 1988). In that model the *rad3-101* mutation leads to an increase in the number of mismatches due to misreplication. These mismatches are then processed by a *RAD1*-dependent step into recombinogenic lesions that are thought to be double-strand-breaks since they require *RAD52* (or *RAD50*) to be processed. Physical evidence for the accumulation of double-strand breaks in a *rad3-101* background supports this notion (MONTELONE, HOEKSTRA and MALONE 1988). While *rad3-101 rad52* double mutants are inviable, *pms1 rad52* double mutants resemble *rad52* single mutants (data not shown). Since we do not see any lethality associated with the *pms1 rad52* double mutant, we suggest that the *pms1* mutation does not lead to the accumulation of a lethal level of double-strand breaks.

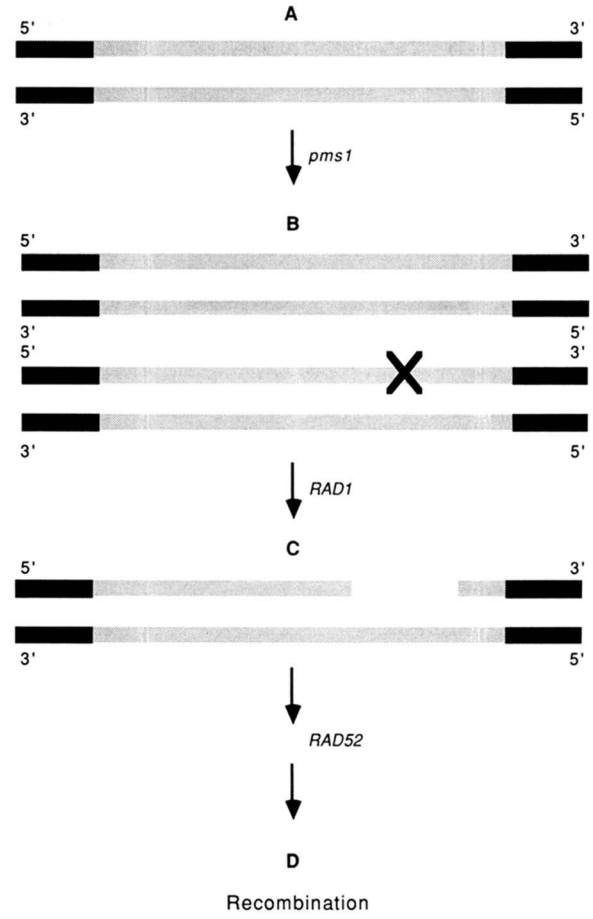

FIGURE 5.—A model explaining the hyperrecombination phenotype in mismatch repair defective *pms1* mutant cells. (A) A prototypical gene is depicted as double stranded DNA prior to DNA replication. (B) DNA replication occurs and mismatches accumulate due to a defect in mismatch repair imposed by the *pms1* mutation. (C) The excision repair machinery, of which *RAD1* is part, recognizes the mismatch and cleaves on either side leaving a single-strand gap. (D) Single-strand gapped DNA either enters directly into the *RAD52*-dependent recombination pathway or is processed into a double-strand break before entering (not shown).

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.

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