MOLECULAR AND CELLULAR BIOLOGY, Nov. 1992, p. 4988–4993
0270-7306/92/114988-06$02.00/0

Vol. 12, No. 11

Genome Rearrangement in *top3* Mutants of *Saccharomyces cerevisiae* Requires a Functional *RAD1* Excision Repair Gene

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Received 28 May 1992/Returned for modification 16 July 1992/Accepted 5 August 1992

***Saccharomyces cerevisiae* cells that are mutated at *TOP3*, a gene that encodes a protein homologous to bacterial type I topoisomerases, have a variety of defects, including reduced growth rate, altered gene expression, blocked sporulation, and elevated rates of mitotic recombination at several loci. The rate of ectopic recombination between two unlinked, homeologous loci, *SAM1* and *SAM2*, is sixfold higher in cells containing a *top3* null mutation than in wild-type cells. Mutations in either of the two other known topoisomerase genes in *S. cerevisiae*, *TOP1* and *TOP2*, do not affect the rate of recombination between the *SAM* genes. The *top3* mutation also changes the distribution of recombination events between the *SAM* genes, leading to the appearance of novel deletion-insertion events in which conversion tracts extend beyond the coding sequence, replacing the DNA flanking the 3′ end of one *SAM* gene with nonhomologous DNA flanking the 3′ end of the other. The effects of the *top3* null mutation on recombination are dependent on the presence of an intact *RAD1* excision repair gene, because both the rate of *SAM* ectopic gene conversion and the conversion tract length were reduced in *rad1 top3* mutant cells compared with *top3* mutants. These results suggest that a *RAD1*-dependent function is involved in the processing of damaged DNA that results from the loss of Top3 activity, targeting such DNA for repair by recombination.**

Topoisomerases are required for a variety of cellular processes, including DNA replication, transcription, recombination, and chromosome condensation and segregation (16, 17). Topoisomerases from several unicellular and multicellular eukaryotes have been identified, including those of the budding yeast *Saccharomyces cerevisiae* (16). The genes encoding topoisomerase I (*TOP1*) and topoisomerase II (*TOP2*) from *S. cerevisiae* have been cloned (5, 6, 14). Recently the gene encoding a putative third topoisomerase from *S. cerevisiae* was identified (15). This gene, *TOP3*, is unique among eukaryotic topoisomerase genes in that it is homologous to bacterial type I topoisomerase genes (e.g., *Escherichia coli topA* and *topB*) but not to other eukaryotic topoisomerase genes (15). The functional homology of the *E. coli topA* gene and *S. cerevisiae TOP3* was demonstrated by the ability of the *topA* gene, when overexpressed, to complement the slow-growth defect of *top3* mutants (15). More recently, Top3 was shown to possess a weak negative supercoil relaxing activity (6a).

Top1 and Top2 have been implicated in the control of mitotic recombination within the rDNA multiple tandem array but not elsewhere in the *S. cerevisiae* genome (4). More recently, *top3* mutants, originally isolated on the basis of their increased rate of recombination between dispersed, repetitive δ elements (15), were also found to have elevated levels of recombination within the rDNA array (4b).

In this report, we demonstrate that *top3* mutants also have an increased rate of ectopic recombination between the unlinked homeologous genes *SAM1* and *SAM2*, while the rate of recombination between these genes is not significantly elevated in either *top1* null or *top2* temperature-sensitive mutants. A novel class of aberrant recombination event that gives rise to genome rearrangements is also observed in *top3* mutants. Altered recombination in the *top3* mutant is at least partially dependent on the presence of a wild-type *RAD1* excision repair gene. The failure to observe altered recombination in the *rad1 top3* double mutant was paralleled by a decreased growth rate. These data suggest that DNA lesions generated in the absence of Top3 require processing by a *RAD1*-dependent excision repair function before fully stimulating recombination. We further suggest that in the absence of excision repair (i.e., in the *rad1 top3* double mutant), lesions persist and result in a decreased growth rate.

MATERIALS AND METHODS

Yeast strains and genetic methods. All yeast strains were derived from W303-1A (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) and W303-1B (*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) (12). The construction of the strains used in the *SAM* ectopic gene conversion assay was described previously, as was the construction of the *rad*:::*LEU2* and *rad52*::*TRP1* derivatives (1a). The construction of the *top1-8*::*LEU2* (RS190) (11) and *top3-3*::*HIS3* (W619-5A) (15) strains was described previously. The *sam1*-Δ*Bgl*II and *sam2*-Δ*Sal*I genes were crossed directly into the *top1* and *top3* mutant backgrounds by using the isogenic wild-type strain W763-2B (*MAT*α *sam1*-Δ*Bgl*II *sam2*-Δ*Sal*I *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) (1a). The *sam* genes were also crossed into the *top2* background by crossing W763-2B with RS191 (*MAT***a** *top2-1 ade2-1 can1-100 his3-11,15 leu2-3,112 tro1-1 ura3-1*) (3), a strain that was the product of a single backcross to W303-1A. Progeny containing both the *sam* genes and the *top2-1* allele were backcrossed three times to W303-1A or W303-1B to obtain *top2-1* strains carrying the *sam* mutation that were at least 93% genetically identical to W303. The *top1-8*::*LEU2* and the *top2-1* strains were kindly provided by Rolf Sternglanz.

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Standard methods for mating, diploid selection, sporulation, and tetrad dissection were employed (11).

Reversion analysis. Single colonies from strains bearing a frameshift mutation at one locus and a deletion-insertion at the other were used to inoculate each of 100 cultures of 5 ml of YPD (1% yeast extract, 2% peptone, 2% dextrose)–0.1 mg of *S*-adenosylmethionine (AdoMet) per ml. The cells were grown to a density of 2×10^7 to 5×10^7/ml at 30°C, centrifuged, and resuspended in 10 ml of YPD. Cultures incubated overnight at 30°C 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 of growth at 30°C was used to determine the rate of prototroph formation by the method of Luria and Delbruck (8). The reversion rate for each frameshift 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 *rad52* and *rad52 top3* strains bearing frameshift mutations at both *SAM* loci was also determined in this manner.

Recombination assay. For most genetic backgrounds, 10-ml YPD cultures supplemented with 0.1 mg of AdoMet per ml were inoculated with single colonies of AdoMet auxotrophic mutants bearing frameshift mutations at both *SAM* loci. The cultures were grown to a density of 5.0×10^7 cells per ml at 30°C. 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 centrifuged and then resuspended in 1 ml of H_2O, and 0.1-ml aliquots were plated onto individual YPD plates to determine the rate of formation of AdoMet prototrophs. After 3 days of incubation at 30°C, the total number of AdoMet prototrophs on the 10 plates was counted, and the frequency of AdoMet prototroph formation was 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 (7).

Growth curve determination. Single wild-type, *rad1*, *top3*, and *rad1 top3* colonies were inoculated into 5-ml YPD cultures and grown to saturation at 28°C. The appropriate volume of preculture was used to inoculate 10-ml YPD cultures to a density of 3.0×10^6 cells per ml, and cultures were then incubated at 28°C. At 30- to 60-min intervals, the density of the culture was determined both by densitometry using a Klett-Summerson meter and by hemacytometer counts.

RESULTS

Elevated levels of ectopic gene conversion in *top3* but not *top1* or *top2* mutants. Recombination rates in *top1*, *top2*, and *top3* mutant strains were assayed with the *SAM* ectopic gene conversion assay (1a). Four-base-pair insertions were made at the 5′ end of *SAM1* and the 3′ end of *SAM2*, creating frameshift mutations that disrupt gene function and make the cells dependent on AdoMet for growth (1a). An AdoMet prototrophic colony appears as the result of a recombination event that replaces the mutation in one gene with wild-type information from the other gene. Recombination between the *SAM* genes is limited to gene conversion because the orientation of the *SAM* genes relative to their centromeres precludes the recovery of reciprocal recombinants (1a). The rate of appearance of AdoMet prototrophs in the *top1*, *top2*, and *top3* strains included in this assay was determined. AdoMet prototrophs appear at a rate 5.8-fold greater than that of the wild type in *top3* mutants but do not appear at rates significantly greater than those of the wild type in *top1* ($\chi^2 = 1.68$, $P = 0.18$) or *top2* ($\chi^2 = 1.69$, $P = 0.20$) strains (Table 1).

TABLE 1. Rates of AdoMet prototroph formation in wild-type and mutant strains

Genotype	Gene conversion rate[a] (events/cell doubling) (10^{-9})	Background reversion rate[b] (10^{-9})
Group A		
Wild type	8.4	0.006
top1	10.0	0.004
top2	12.4	0.006
top3	47.8	0.006
rad1	6.3	0.008
rad1 top3	13.8	0.010
Group B		
rad52	0.02	0.040
rad52 top3	0.03	ND[c]

[a] Gene conversion rates were determined by the method of the median for group A (13) and by fluctuation analysis for group B (14). The wild-type, *rad1*, and *rad52* rates were determined previously (10). All assays were performed at 30°C.

[b] Background reversion rates are the sums of the rates determined for both *sam* genes by fluctuation analysis (14).

[c] ND, not determined.

The 4-bp insertions disrupting *SAM1* and *SAM2* lead to changes in restriction endonuclease cleavage sites that allow us to determine the nature of the event leading to AdoMet prototrophy. Genomic DNA from a single prototrophic colony from each trial was digested simultaneously with either *Bgl*II and *Sal*I or *Cla*I and *Sal*I and blotted to nitrocellulose (1a). In all strains, more than 84% of the prototrophs had regained the *Sal*I site at *SAM2*, indicative of repair of the 4-bp insertion in *sam2-ΔSal*I by gene conversion using the corresponding wild-type information from the *sam1-ΔBgl*II gene (data not shown). Loss of the *Cla*I site from *sam1-ΔBgl*II as a result of gene conversion of the insertion mutation by wild-type information from *sam2-ΔSal*I occurred much less frequently. This bias favoring the conversion of *sam2-ΔSal*I is likely a result of the 4-bp insertion being adjacent to the longest stretch of uninterrupted sequence identity between *SAM1* and *SAM2* (1a).

Significant levels of gene conversion are observed only when a wild-type copy of the double-strand break repair gene *RAD52* is present (Table 1). The rate of prototroph formation in *rad52 top3* double mutant cells is approximately equal to the rate observed in *rad52* single mutants, which is at or below the combined reversion rates for *sam1-ΔBgl*II and *sam2-ΔSal*I in these cells (Table 1).

Aberrant recombinants in *top3* mutants have conversion tracts that include extensive lengths of nonhomologous sequence. The incomplete identity between the *SAM1* and *SAM2* genes provides a convenient tool for determining the extent of information exchange following a gene conversion event. The length of a gene conversion tract can be estimated by determining, with genomic DNA blots, the presence or absence of a number of polymorphic restriction sites adjacent to the repaired mutation (Fig. 1A). Conversion events that include more than the site of mutation are called coconversions. It was previously shown that in the majority of wild-type *SAM2* recombinants (73%), only the mutated *Sal*I site in *sam2-ΔSal*I is converted (1a). Among the fraction of *SAM2* recombinants obtained in wild-type strains that have undergone coconversion (27%), none acquired the

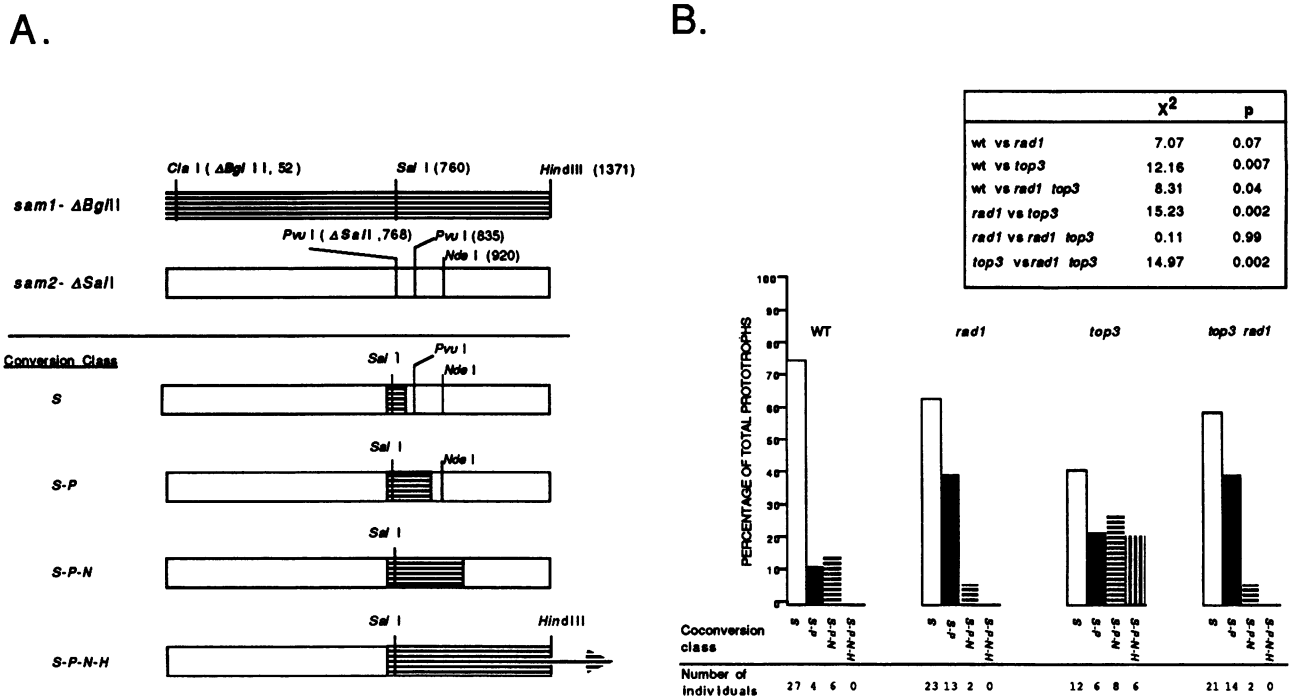

FIG. 1. Conversion tract length and distribution after gene conversion. (A) DNA was isolated from cultures of the AdoMet prototrophs that contain recombinant *SAM2* genes and was digested with *Bgl*II and *Pvu*I, *Nde*I, or *Hin*dIII and blotted as described previously (1a). The 460-bp *Eco*RV-*Sal*I *SAM1* and the 468-bp *Eco*RV-*Sal*I *SAM2* DNA fragments, labeled with ^{32}P by nick translation, were used as probes as described previously (1a). The parental mutant *sam* genes are pictured at the top of the diagram. The *sam1*-Δ*Bgl*II sequence is represented by the striped bar. The *sam2*-Δ*Sal*I sequence is represented by the open 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 the donated *sam1*-Δ*Bgl*II sequence surrounding only the *Sal*I site. *S-P* coconversions are missing the *Pvu*I site and are depicted with the *sam1*-Δ*Bgl*II sequence extending past the *Pvu*I position. *S-P-N* coconversions are missing both the *Pvu*I and the *Nde*I sites and are depicted with the *sam1*-Δ*Bgl*II sequence extending past the *Nde*I position. *S-P-N-H* coconversions have lost the *Pvu*I and *Nde*I sites and have also gained the *Hin*dIII site at the end of the *sam1*-Δ*Bgl*II sequence. The conversion tract is shown extending past the end of the gene into sequences 3′ to the *SAM1* locus. (B) Conversion tract length distribution in wild-type (wt) and mutant backgrounds was determined by analyzing all of the recombinant *SAM2* genes resulting from gene conversion in recombination-proficient strains. The number of recombinants in each coconversion class (*S*, *S-P*, *S-P-N*, and *S-P-N-H*) was determined and was expressed as the percentage of the total number of prototrophs of that genetic background that were analyzed. The insert shows the results of a contingency χ^2 analysis (two by four) of the data. The wild-type and *rad1* data were published previously (10).

*Hin*dIII site at the 3′ end of the *SAM1* coding sequence, indicating that the conversion tracts do not extend into the 3′-flanking DNA (Fig. 1). In addition, when the *Nde*I site normally flanking the *Sal*I site in *SAM2* is lost during coconversion, the proximal *Pvu*I site is also lost, indicating that the tract of *SAM1* information converting *sam2*-Δ*Sal*I to *SAM2* is always continuous (1a).

The pattern of coconversion in *top3* cells is both quantitatively and qualitatively different from that found in wild-type cells. In *top3* cells, the majority of *SAM2* conversions (62.5%) (Fig. 1) are coconversions, indicating that conversion tracts are longer in *top3* mutant cells than in wild-type cells. In addition, a novel coconversion event is observed (19%) (Fig. 1) in which the tract of information imported from *sam1*-Δ*Bgl*II extends beyond the *SAM1* coding sequence, incorporating the *Hin*dIII site at the termination codon of the *SAM1* gene into the converted *SAM2* gene (Fig. 1, *S-P-N-H*). More than 1,500 bp of DNA flanking the *SAM2* gene was deleted in these recombinants (Fig. 2 and data not shown). Further analysis of these recombinants by genomic DNA blotting (data not shown) showed that the conversion tracts extend between 1 to 7 kb beyond the *SAM1* termination codon (Fig. 2). As found previously for wild-type recombinants, all of the *top3* conversion tracts are continuous. Because none of the 9.5 kb of DNA flanking the 3′ end of the *SAM1* gene hybridizes with any other genomic sequences on low-stringency genomic blots (data not shown), these extended conversion tracts could not have terminated in repetitive Ty or δ elements, the *S. cerevisiae* retrotransposon and its long terminal repeat (2).

The altered recombination phenotype of *top3* mutants is dependent on a wild-type *RAD1* excision repair gene. In previous work, we established that a functional *RAD1* gene is required for the elevated rate of recombination between the *SAM* genes in mismatch repair-defective *pms1* null mutants (1a). This result prompted us to examine the effect of the *rad1* mutation on the elevated recombination phenotype of the *top3* gene. We previously showed that while a null mutation in the *rad1* excision repair gene alone has no significant effect on the rate of recombination between the *SAM* genes, the distribution of conversion tract lengths is significantly different from that seen in wild-type cells (Fig. 1). When *sam* ectopic gene conversion is assayed in *rad1* *top3* double mutants, we find that the recombination rate is

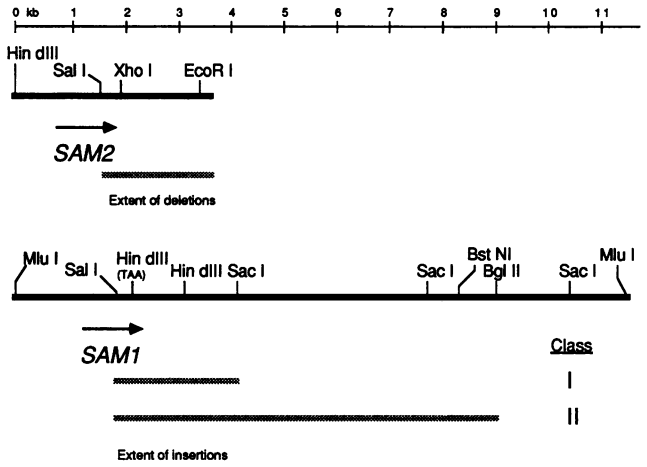

FIG. 2. Description of the *S-P-N-H* coconversions. The *S-P-N-H* recombinants described in the legend to Fig. 1 and the text were further analyzed by genomic blot analysis. A restriction map of the area flanking the 3′ ends of the *SAM1* and *SAM2* genes that lies within our genomic clones is depicted (13). DNA fragments that together span the pictured 3′-flanking regions of *SAM1* and *SAM2* were used as hybridization probes to determine the extent of the deletion of *SAM2* information and the insertion of *SAM1* information in the recombinants. In all six of the *S-P-N-H* coconversions, the 1.5 kb of DNA normally flanking the 3′ end of the *SAM2* gene is deleted. These deletions may be larger, because only 1.5 kb of the *SAM2* 3′ flanking DNA has been cloned (13). The recombinants are separated into classes on the basis of the amount of *SAM1* 3′-flanking DNA that is inserted into *SAM2*. The one class I coconversion has inserted at least 1 kb of *SAM1* 3′-flanking DNA into *SAM2*. The five class II coconversions were all found to have insertions that terminate in the same 750-bp span of DNA (between the *Bst*NI and *Bgl*II sites) 6 kb downstream from the termination codon of *SAM1*. These insertions could be up to 7.5 kb in length.

reduced from that found in *top3* mutants but is still significantly above the level observed in *rad1* mutants (2.2-fold, $\chi^2 = 14.3$, $P = 0.002$) (Table 1). Interestingly, the *rad1 top3* coconversion pattern is almost identical to the pattern seen in *rad1* single mutant cells ($\chi^2 = 0.11$, $P = 0.99$) (Fig. 1), indicating that conversion tracts are significantly shorter in these cells than in the *top3* single mutants. The aberrant recombinants in which conversion tracts terminate outside of the *SAM* coding sequence that had been observed in *top3* cells are not observed in *rad1 top3* double mutants. These epistatic relationships suggest that while wild-type Rad1 function is required for the increased conversion tract length in *top3* single mutant cells, there is only a partial requirement for Rad1 function for an elevated recombination rate.

TABLE 2. Growth rates of wild-type and mutant cells

Genotype	Doubling time (min)[a]	% of wild-type rate
Wild type	72	100
rad1	72	100
top3	160	45
rad1 top3	343	21

[a] Cells were grown in YPD medium at 28°C as discussed in Materials and Methods. Cell density was determined by hemacytometer counts and by colorimetry by using a Klett-Summerson colorimeter.

The *rad1* and *top3* mutations have a synergistic effect on cell growth rate. With crosses between the *rad1* and *top3* single mutant strains, we noted three distinct spore colony sizes (Fig. 3). Experiments to determine growth rate revealed that while *top3* mutants grow at 45% of the wild-type rate, the *rad1 top3* double mutants grow at 21% of the wild-type rate (Table 2). The *rad1* mutation alone has no effect on the growth rate. Therefore, the *rad1* and *top3* mutations have a synergistic effect on the growth rate of the cell.

DISCUSSION

For this report, we have used an ectopic gene conversion assay to show that the rate of recombination between the naturally duplicated but unlinked genes *SAM1* and *SAM2* is increased nearly sixfold in *top3* mutant cells (Table 1). The loss of Top3 function also results in an elevated level of recombination at several other loci, including those in the rDNA tandem array (14a, 15). While mutations in the *TOP1* and *TOP2* genes also increase rDNA recombination, they do not affect recombination between the *SAM* genes or at other loci (4) (Table 1). Therefore, the wild-type Top3 protein appears to have a role in mitotic recombination that is distinct from the roles of Top1 and Top2.

We have also found that the *top3* mutation increases the incidence of coconversion substantially (62.5 versus 25% in wild-type cells) (Fig. 1). Even more striking is the appearance of aberrant *SAM2* recombinants in which conversion

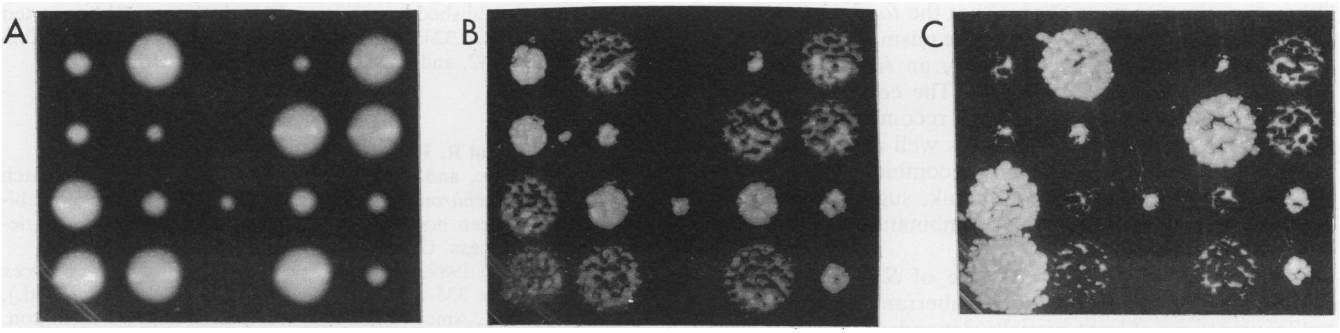

FIG. 3. Spore colony phenotypes resulting from *rad1*::*LEU2* × *top3*::*HIS3* crosses. The *rad1*::*LEU2* and *top3*::*HIS3* single mutant strains were crossed, and the resulting diploids sporulated and were dissected onto YPD agar. (A) After 4 days of growth at 30°C, three spore colony sizes were noted. (B) Spore colonies were replica plated to histidineless medium to determine which contained the *top3*::*HIS3* allele. (C) Spore colonies were also replica plated to leucineless medium to determine which contained the *rad1*::*LEU2* allele. The largest colonies were either wild-type or *rad1*::*LEU2* single mutants, the medium-sized colonies were *top3*::*HIS3* single mutants, and the smallest colonies were *rad1*::*LEU2 top3*::*HIS3* double mutants.

tracts extend beyond the coding sequence, replacing information deleted from the *SAM2* gene and its 3′ flanking sequence with information from *SAM1* and its 3′ flanking region (Fig. 1, *S-P-N-H*). There is no nucleotide sequence identity at least 130 bp 3′ to the *SAM1* and *SAM2* coding sequences (13).

The aberrant coconversion events comprise a significant fraction of the coconversions in *top3* mutant cells (15.8%) but are not observed in wild-type cells (Fig. 1). A detailed genomic blot analysis (data not shown) of these recombinants revealed that five of them had deleted at least 1.5 kb of DNA flanking the 3′ end of *SAM2* while inserting up to 7 kb of DNA flanking the 3′ end of *SAM1* (Fig. 2). These conversion tracts are always continuous (data not shown). We noted previously that coconversion during recombination between the *SAM* genes is unaffected by the mismatch repair defect in *pms1* mutant cells, suggesting that extensive heteroduplex formation and mismatch correction are unlikely to account for coconversion (1a). Mismatch repair of heteroduplex DNA is also unlikely to account for the incorporation of up to 7.0 kb of nonhomologous DNA in the exceptional coconversions in *top3* mutants. This suggests that loss of Top3 activity in *top3* mutants may increase coconversion by promoting the formation of double-strand gaps, some of which extend into regions of nonidentical sequence. Recent work has shown that as little as 4 bp of sequence identity can promote targeted integration of a DNA fragment into the *S. cerevisiae* genome (9). Accordingly, we suggest that double-strand gaps formed in the *top3* mutant cells might be repaired as the result of pairing between short homologous sequences 3′ to both *SAM1* and *SAM2*. These sequences must be smaller than the 50 bp necessary to generate a hybridization signal on a low-stringency genomic blot (data not shown), because no duplicate signals were generated with probes from over 9 kb of DNA flanking the 3′ end of *SAM1* (Fig. 2). We are determining whether such stretches of identical DNA sequence exist by comparing the sequences at the *SAM1-SAM2* junctions in the long-tract recombinants with the corresponding sequences flanking *SAM1* and *SAM2*.

The hyperrecombination and genome rearrangement observed in *top3* mutants could result from changes at any of several steps in the recombination process. Recently we have observed similar increases (7.3-fold) in the rate of ectopic recombination between identical *SAM1* genes in *top3* mutants (1). This similarity suggests that the *top3* mutation is enhancing the rate of recombination at a step, such as initiation, when sequence identity is not crucial. Other experiments have shown that the *top3* mutation does not affect recombination between the mismatched *SAM1* and *SAM2* genes when it is initiated by an *HO* endonuclease-catalyzed double-strand break (1). The equivalent effect of the *top3* mutation on spontaneous recombination between identical and mismatched genes, as well as the absence of any effect of the *top3* mutation on recombination subsequent to initiation by a double-strand break, suggests that loss of Top3 activity may increase recombination by increasing initiation.

Both the stimulation of the rate of *SAM* ectopic gene conversion and the appearance of aberrant recombinants in *top3* mutants are at least partially dependent on the presence of a wild-type *RAD1* excision repair gene (Table 1; Fig. 1). These results suggest that a wild-type *RAD1* gene may be required to obtain fully elevated rates of spontaneous initiation in *top3* mutant cells. This increase in initiation could be due to *RAD1*-dependent processing of DNA lesions that accumulate in cells lacking Top3 activity. Alternatively, as has been suggested previously, Rad1 may act after initiation (10). Recent work has shown that loss of Rad1 function results in a substantial reduction in the rate of recombination following initiation by a double-strand break (1, 4a). Therefore, loss of Rad1 function may affect recombination at any of several steps during recombination in *top3* mutant cells. Regardless of when Rad1 is acting, the synergistic effect of the *rad1* and *top3* mutations on the growth rate suggests that lesions deleterious to the cell accumulate in *rad1 top3* double mutants (Table 2). The dependence of *SAM* ectopic gene conversion on the presence of a wild-type copy of the *RAD52* gene in *top3* cells argues that *RAD1*-dependent processing of lesions facilitates further processing by *RAD52*-dependent recombination. The viability of the *rad52 top3* double mutants, however, suggests that the *top3* mutation does not lead to a lethal level of double-strand breaks.

The appearance of the aberrant recombinants in the *top3* mutant cells in this study parallels previous work with *top3* mutants in which an increased rate of deletion formation by intramolecular recombination between repeated sequences was observed (15). These results strongly suggest that the *TOP3* gene is important for the maintenance of genomic integrity. The restoration of wild-type recombination in the *rad1 top3* double mutant argues that excision repair is required for genome rearrangements to occur in *top3* cells and, therefore, that a functional *TOP3* gene prevents such genomic damage. Although their effect on recombination is restricted to the rDNA array, the wild-type function of the *TOP1* and *TOP2* genes is also required to preserve the integrity of the genome at this locus (4). Therefore, one function of the topoisomerases may be to prevent recombination between repeated sequences from constantly rearranging the genome. This function of DNA topoisomerases may have played an important role in the evolution of complex eukaryotic genomes.

ACKNOWLEDGMENTS

We thank Lorraine Symington, Hamish Young, and David Shore for helpful discussions and for critically reading the manuscript. We also thank the members of the Rothstein laboratory and Oliver Jovanovic for their helpful comments on the manuscript and discussions during this study. We gratefully acknowledge Rolf Sternglanz for providing the *top1* and *top2* strains. We also thank Jacqueline Fishman-Lobell, Jim Haber, Raymond Kim, and Jim Wang for communicating experimental results prior to publication.

A.M.B. was supported by National Institutes of Health (NIH) postdoctoral fellowship GM-12079-02. R.R. was an American Heart Association Established Investigator. This work was also supported by NIH grants GM 324587 and CA 21111, NSF grants DCB 870383 and DMB 8919182, and a grant from the Irma T. Hirschl Trust.

REFERENCES

1. **Bailis, A., and R. Rothstein.** Unpublished data.
1a. **Bailis, A. M., and R. Rothstein.** 1990. A defect in mismatch repair in *Saccharomyces cerevisiae* stimulates ectopic recombination between homeologous genes by an excision repair dependent process. Genetics **126**:535–547.
2. **Boeke, J. D.** 1989. Transposable elements in *Saccharomyces cerevisiae*, p. 335–374. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
3. **Brill, S. J., and R. Sternglanz.** 1988. Transcription-dependent DNA supercoiling in yeast DNA topoisomerase mutants. Cell **54**:403–411.
4. **Christman, M. F., F. S. Dietrich, and G. R. Fink.** 1988. Mitotic recombination in the rDNA of *Saccharomyces cerevisiae* by the

combined action of DNA topoisomerases I and II. Cell **55**:413–425.

4a. **Fishman-Lobell, J., and J. Haber.** In press.

4b. **Gangloff, S., and R. Rothstein.** Unpublished data.

5. **Goto, T., and J. C. Wang.** 1984. Yeast DNA topoisomerase II is encoded by a single-copy, essential gene. Cell **36**:1073–1080.

6. **Goto, T., and J. C. Wang.** 1985. Cloning of yeast *TOP1*, the gene encoding topoisomerase I, and the construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. Proc. Natl. Acad. Sci. USA **82**:7178–7182.

6a. **Kim, R., and J. Wang.** Submitted for publication.

7. **Lea, D. E., and C. A. Coulson.** 1949. The distribution in the numbers of mutants in bacterial populations. J. Genet. **49**:264–285.

8. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.

9. **Schiestl, R. H., and T. D. Petes.** 1991. Integration of DNA fragments by illegitimate recombination in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88**:7585–7589.

10. **Schiestl, R. H., and S. Prakash.** 1988. *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. **8**:3619–3626.

11. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

12. **Thomas, B. J., and R. Rothstein.** 1989. The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of *rad1* and *rad52* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Cell **56**:619–630.

13. **Thomas, D., R. Rothstein, N. Rosenberg, and Y. Surdin-Kerjan.** 1988. *SAM2* encodes the second methionine *S*-adenosyl transferase in *Saccharomyces cerevisiae*: physiology and regulation of both enzymes. Mol. Cell. Biol. **8**:5132–5139.

14. **Thrash, C., A. T. Bankier, B. G. Barrell, and R. Sternglanz.** 1985. Cloning, characterization, and sequence of the yeast DNA topoisomerase I gene. Proc. Natl. Acad. Sci. USA **82**:4374–4378.

14a. **Wallis, J., L. Arthur, S. Gangloff, and R. Rothstein.** Unpublished data.

15. **Wallis, J. W., G. Chrebet, G. Brodsky, M. Rolfe, and R. Rothstein.** 1989. A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. Cell **58**:409–419.

16. **Wang, J. C.** 1985. DNA topoisomerases. Annu. Rev. Biochem. **54**:665–697.

17. **Wang, J. C., P. Caron, and R. Kim.** 1990. The role of DNA topoisomerases in recombination and genome stability: a double-edged sword? Cell **62**:403–406.