SGS1, a Homologue of the Bloom's and Werner's Syndrome Genes, Is Required for Maintenance of Genome Stability in *Saccharomyces cerevisiae*

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ABSTRACT

The *Saccharomyces cerevisiae SGS1* gene is homologous to *Escherichia coli* RecQ and the human BLM and WRN proteins that are defective in the cancer-prone disorder Bloom's syndrome and the premature aging disorder Werner's syndrome, respectively. While *recQ* mutants are deficient in conjugational recombination and DNA repair, Bloom's syndrome cell lines show hyperrecombination. Bloom's and Werner's syndrome cell lines both exhibit chromosomal instability. *sgs1*Δ strains show mitotic hyperrecombination, as do Bloom's cells. This was manifested as an increase in the frequency of interchromosomal homologous recombination, intrachromosomal excision recombination, and ectopic recombination. Hyperrecombination was partially independent of both *RAD52* and *RAD1*. Meiotic recombination was not increased in *sgs1*Δ mutants, although meiosis I chromosome missegregation has been shown to be elevated. *sgs1*Δ suppresses the slow growth of a *top3*Δ strain lacking topoisomerase III. Although there was an increase in subtelomeric Y′ instability in *sgs1*Δ strains due to hyperrecombination, no evidence was found for an increase in the instability of terminal telomeric sequences in a *top3*Δ or a *sgs1*Δ strain. This contrasts with the telomere maintenance defects of Werner's patients. We conclude that the *SGS1* gene product is involved in the maintenance of genome stability in *S. cerevisiae*.

MUCH of our knowledge of the enzymes involved in catalyzing the key events in genetic recombination has come from an analysis of mutants of bacteria or yeasts displaying an altered frequency of recombination. The most extensively characterized recombination-deficient mutants have been derived from *Escherichia coli*, in which several overlapping recombination pathways have been defined by genetic studies (reviewed in WEST 1992, 1994). Less is known about the protein constituents of recombination pathways in eukaryotes, although a substantial number of genes involved in mitotic and/or meiotic recombination have been identified in both budding and fission yeast. While most of these yeast genes show no significant primary sequence similarity to their *E. coli* counterparts, there are notable exceptions, such as the *Saccharomyces cerevisiae* Rad51 and Dmc1 proteins, which appear to be structural and functional homologues of *E. coli* RecA protein (BISHOP *et al.* 1992; SHINOHARA *et al.* 1992).

The product of the *E. coli recQ* gene is the prototypical member of a family of highly conserved DNA helicases presumed to participate in recombination processes (NAKAYAMA *et al.* 1984). Homologues of RecQ have been identified in budding yeast (Sgs1p) and in human cells (the RECQL, BLM and WRN proteins) (GANGLOFF *et al.* 1994; PURANAM and BLACKSHEAR 1994; SEKI *et al.* 1994; ELLIS *et al.* 1995; WATT *et al.* 1995; YU *et al.* 1996). The *SGS1* gene was isolated independently in two laboratories, although in each case in conjunction with studies on DNA topoisomerases. A mutant allele of *SGS1* was identified as a suppresser of the slow-growth phenotype of *top3* mutants deficient in topoisomerase III, and Sgs1p was shown subsequently to interact with topoisomerase III in *S. cerevisiae* (GANGLOFF *et al.* 1994). We identified *SGS1* during a search for proteins that could interact with topoisomerase II protein, and we have shown previously that *sgs1* strains display a reduction in the fidelity of chromosome segregation during both mitotic and meiotic cell divisions (WATT *et al.* 1995). The *SGS1* gene was already in the databases under the designation *TPS1* (GENEMB7870 deposited by R. STERNGLANZ) and was isolated in this case by virtue of genetic interactions with topoisomerase I (R. STERNGLANZ, personal communication).

BLM, one of three human *recQ*-like genes, has been identified recently as the gene defective in individuals suffering from the cancer-prone condition, Bloom's syndrome (ELLIS *et al.* 1995). Cell lines derived from Bloom's syndrome individuals display a high degree of genomic instability, including an elevated frequency of recombination between both sister chromatids and homologous chromosomes (GERMAN 1974). Although significant sequence similarity is evident within the consensus helicase domains present in the RecQ and BLM proteins, the predicted size of the BLM protein is 159

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kDa compared to 63 kDa for RecQ, suggesting that the BLM protein may perform one or more roles not performed by its bacterial homologue. In contrast, the BLM and Sgs1 proteins differ in length by only 30 residues, and the structural similarity between Sgs1p and BLM includes a serine-rich, highly charged 575-amino-acid N-terminal domain that is absent from both the *E. coli* RecQ and human RECQL proteins (ELLIS *et al.* 1995; see WATT and HICKSON 1996 for a review of the members of this protein family). Similarly, the WRN protein is 162 kDa, differs in length from Sgs1p by only 16 residues, and has a highly charged N-terminal domain. *SGS1* is the only structural homologue of *BLM* and *WRN* in *S. cerevisiae* and may be a functional homologue of one or both human genes.

To compare the phenotype of a yeast strain lacking functional Sgs1p with those of *E. coli recQ* mutants and Bloom's and Werner's syndrome cell lines, we deleted a portion of the *SGS1* gene in a variety of genetic backgrounds. We found that *sgs1*Δ strains, like Bloom's syndrome cell lines, show an elevated frequency of mitotic recombination between homologous sequences. Unlike Werner's syndrome, *sgs1*Δ strains do not have any telomere maintenance defects. Other phenotypes similar to Werner's were not tested. We compare and contrast the phenotypes of cells deficient in the different members of the RecQ family of helicases and discuss possible ways in which helicases and topoisomerases might cooperate to maintain genomic stability in eukaryotes.

MATERIALS AND METHODS

Plasmids and *S. cerevisiae* strain construction: Gene disruptions (one-step gene transplacements) (ROTHSTEIN 1983) and plasmid integrations into the yeast genome were achieved using linearized plasmid DNA and the modified lithium acetate transformation protocol of GIETZ *et al.* (1992).

The plasmid pPWΔSGS1 (WATT *et al.* 1995) was used to disrupt *SGS1* with *LEU2* (deleting from *Hpa*I to *Eco*RV in the coding sequence) using restriction enzymes *Nco*I and *Pst*I in all of the strain backgrounds. *sgs1*Δ strains were identified by Southern analysis. In some cases the *LEU2* gene used in *SGS1* disruptions was disrupted with *LYS2* using pRHB142. This is a pGEM3zf(-) vector with the 2230-kb *Xho*I-to-*Sal*I *LEU2* fragment at the *Sal*I site. The *LYS2* gene was inserted at the *Cla*I site within the *LEU2* coding sequence. Correct replacement of the markers was checked genetically by screening Lys^+ transformants for a Leu^- phenotype.

TOP3 was disrupted using plasmid pWJ258, kindly provided by Dr. R. ROTHSTEIN, using *Not*I. The disruption contains the *LEU2* gene. Transformants were checked by Southern analysis as well as by PCR using primers flanking the *TOP3* gene and within *LEU2*. *top3*Δ was also confirmed genetically by the ability of the *sgs1*Δ disruption to rescue the slow growth of the *top3*Δ strains (GANGLOFF *et al.* 1994). This also confirmed the *sgs1*Δ phenotype.

*rad1*Δ and *rad52*Δ were generated using plasmids pL962 obtained from Dr. R. KEIL (KEIL and MCWILLIAMS 1993) and pSM20 obtained from Dr. D. SCHILD (SCHILD *et al.* 1983), respectively. Transformants were confirmed by Southern analysis and checked for UV and γ-ray sensitivity, respectively.

The plasmids pJH257 and pRHB152 contain a single copy of *MAT***a** in pBR322 between the *Eco*RI and *Hin*dIII sites. *URA3* is inserted at the *Ava*I site (BORTS and HABER 1987). pRHB152 is the same with *CYH2* at the *Nru*I site of pBR322 in addition. These were integrated into *MAT***a** strains using restriction enzyme *Bgl*II to create a *MAT***a**-*URA3*-*MAT***a** structure or integrated at the opposite mating type using *Bgl*II and *Pvu*II to create *MATα*-*URA3*-*MATα* as described previously (BORTS *et al.* 1984).

The Y′ ::*URA3*-*SUP11* structures at the right end of chromosome *XV* and the left end of chromosome *IX* were created using pEL2 (LOUIS and HABER 1989). Strains containing these marked chromosomes have been used in previous recombination studies and are well characterized (LOUIS and HABER 1990; LOUIS *et al.* 1994).

The necessary combinations of gene disruptions and markers were obtained by genetic crosses using an isogenic series of strains with the appropriate backgrounds. Strains with multiple disruptions using *LEU2* were confirmed by Southern analysis.

Growth of microorganisms and DNA manipulation: Growth of *E. coli* and yeast and standard recombinant DNA techniques were as described previously (SAMBROOK *et al.* 1989; SHERMAN and HICKS 1991; SHERMAN 1991; AUSUBEL *et al.* 1994). CHEF gel electrophoresis and Southern blotting methods were essentially as described in SAMBROOK *et al.* (1989) and AUSUBEL *et al.* (1994).

Mitotic recombination (loss) of *URA3* from a *MAT*-*MAT* interval: Haploid strains PW10 and PW20 and the corresponding *sgs1*Δ strains were cultured in SC medium lacking uracil. Single colonies were resuspended in water and plated onto nonselective YPD medium. From the YPD media, individual colonies of each strain were resuspended in water and dilutions were plated on SC medium with and without 5-fluoroorotic acid (5-FOA) at 1 mg/ml. Dilutions were chosen such that between 50 and 500 colonies were counted on each plate. The rate of loss of the *URA3* and vector sequences between the flanking *MAT*s was calculated using the median method of LEA and COULSON (1949). At least 24 colonies were analyzed.

In the isogenic series of YP1 strains in which *CYH2* is also in the interval between flanking *MAT* loci, a similar calculation of marker loss rates was performed. The strains had the recessive resistant $cyh2^R$ mutation at the normal locus with the dominant *CYH2* gene in the *MAT* interval, which therefore confers sensitivity to cyclohexamide. In this case, screens for loss of the interval were performed by plating dilutions of resuspended colonies on YPD medium containing cyclohexamide. In one experiment, measurements were done as described above on 16 colonies for each genotype, with dilutions plated on whole plates. In a second experiment, 16 colonies for each genotype were used in the calculation and 10-μl drops of the serial dilutions were plated in an array. Dilutions that resulted in five to 50 colony forming units per drop were used for counting.

Recombination (intrachromosomal and ectopic) involving *URA3* embedded in subtelomeric Y′ sequences: The Y′ ::*URA3*-*SUP11* structure has been used in several recombination assays (LOUIS and HABER 1990; LOUIS *et al.* 1994). Loss of *URA3* can occur by recombination with other Y′ elements (interchromosomal ectopic interactions; see Figure 2, A and B for details) or by recombination between the flanking $TG_{1\text{-}3}$ sequences (intrachromosomal deletion; see Figure 2, C and D for details). The rate of *URA3* loss was measured for two marked Y′s, one with flanking $TG_{1\text{-}3}$ at the right end of chromosome *XV* and the second without the internal tract of $TG_{1\text{-}3}$ at the left end of chromosome *IX*. The loss rate was calculated by the median method as described above on 24 colonies for each genotype. Whole colonies were suspended in water and serial 10-fold

dilutions were made. Ten-microliter drops of each dilution were plated on both SC and 5-FOA media such that five to 50 colony forming units were countable in the appropriate dilution.

Mitotic heteroallelic recombination: Freshly constructed PWD80, PWD80DSGS1, PWD80ΔRAD52 and PWD80ΔSGS1-ΔRAD52 diploids were plated on nonselective YPD media. Individual colonies were then resuspended in water and dilutions were plated on SC −methionine, SC −lysine and SC media. Colony numbers were analyzed according to the median method described above. The tests were repeated four times with seven to 15 colonies resuspended for each test of a given strain.

Statistical analysis of mitotic recombination: Statistical comparisons between different strains within an isogenic series, for example *SGS1 vs. sgs1Δ* or *rad52ΔSGS1 vs. rad52Δsgs1Δ* for the *MAT-MAT* interval, were made by rank order (see for example WIERDL *et al.* 1996). The frequencies for each colony of the two strains being compared (equal numbers of colonies are used) were converted to rates using the median method equation (LEA and COULSON 1949) and ranked together. A chi-squared analysis was performed to test whether one strain had significantly more colonies ranked in the top half of the rate values than the other strain. If the two strains had equal rates, then the expected number of colonies in the upper half of the rank order for a given strain is half of the colonies. A chi-squared value of >3.85 ($P < 0.05$) indicates a significant deviation from the expected 50% and therefore a significant difference in rates between the strains.

Sporulation, spore viability, and meiotic recombination: The diploid yeast strains PWD80 (*SGS1*) and PWD80ΔSGS1 (*sgs1Δ*) were sporulated and asci were dissected as described in SHERMAN and HICKS (1991). Dissected spores were scored as viable if they produced a visible colony after 3 days of growth on YPD agar. Meiotic recombination was assayed at two heteroallelic loci using random spore analysis. Random spore analyses were performed as previously described (BORTS *et al.* 1986) with the sporulated culture treated with glusulase, sonicated, and then plated on canavanine-containing medium as well as on SC −methionine +canavanine and SC −lysine +canavanine media. The rate of meiotic recombination was calculated from the ratio of Met^+ Can^R/Can^R and Lys^+ Can^R/Can^R.

The diploids Y55-D106, Y55-D107 and RHB2180 were sporulated and asci were dissected. Those tetrads with three and four viable spores were scored for marker configurations at *HIS4, MAT* and a centromere. Ditypes and tetratypes of the *sgs1Δ* strains (Y55-D106 and Y55-D107) were compared with those of RHB2180 (an isogenic *SGS1* strain) for the *HIS4-CEN* and *CEN-MAT* intervals.

Diploids Y55-D108 and Y55-D109 were sporulated and random spores were selected on SC −uracil −tryptophan +cyclohexamide medium. These strains are isogenic with the strains previously used for studying missegregation of chromosome *IV* in *sgs1Δ* strains (WATT *et al.* 1995). In addition to the marked centromeres of chromosome *IV* allowing for the identification of meiosis I missegregations, *ade8* segregates >200 cM distal to the centromere. The disomic random spores were scored for adenine auxotrophy. If there were no relationship between recombination on chromosome *IV* and the missegregation of *IV,* 25% of the disomic spores would be Ade^-.

Telomere and subtelomere structure: The structure of the telomeres in *sgs1Δ* and *top3Δ* strains were assessed by Southern analysis. Genomic DNA from *SGS1 TOP3, sgs1Δ TOP3, SGS1 top3Δ*, and *sgs1Δ top3Δ* strains was digested with *Xho*I and the resultant fragments were separated on a 0.8% agarose gel. The most distal *Xho*I site at many chromosome ends is ~1 kb from the terminus, making it ideal for assessing changes in the ~350-bp $TG_{1\text{-}3}$ telomere tract. Long-term (>100 generations) and short-term (~20 generations) cultures of genotyped spore colonies were tested. Probing with pEL30, which contains $TG_{1\text{-}3}$ and Y′ sequences, was used to assess the length of the $TG_{1\text{-}3}$ tracts as well as the general structure of the telomere.

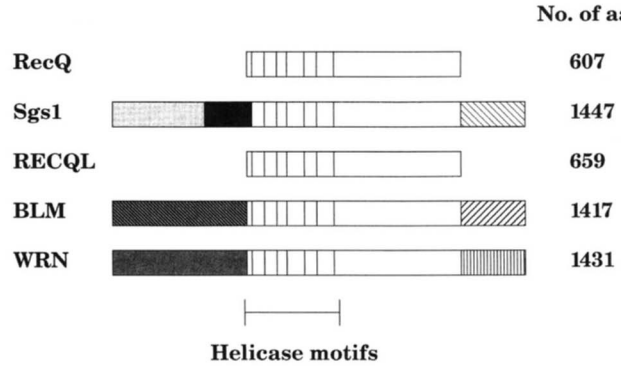

FIGURE 1.—Schematic representation of members of the RecQ family of helicases (as indicated on the left). The proteins are aligned by the positions of the seven helicase motifs, which are shown as vertical lines and are labeled below. The N-terminal and C-terminal domains unique to the Sgs1, BLM and WRN proteins are shown with different shading patterns. The N-terminus of each of these are similar in composition (serine rich and highly charged). ■, the region of Sgs1p that interacts with topo II *in vivo.* The number of amino acid residues in each protein is shown on the right.

RESULTS

***SGS1*, a yeast homologue of the *E. coli recQ* and human *BLM* and *WRN* genes:** Figure 1 shows a schematic representation of the structural relationship between the five known members of the RecQ family of proteins. The most highly conserved regions of these five proteins correspond to the seven "signature" motifs found in a wide variety of DNA and RNA helicases (GORBALENYA *et al.* 1989; KOONIN 1991). The helicase subfamily to which the Sgs1 protein belongs contains the "DEAH" box in motif II (Figure 1). The region of Sgs1p that interacts with topoisomerase II is indicated in Figure 1.

To investigate the roles of Sgs1p *in vivo,* mutant strains were constructed in which a large portion of the *SGS1* gene had been deleted (see Table 1 for a list of strains used). The construct used to make the *SGS1* deletion contains the *LEU2* gene inserted after codon 407 and includes the removal of 540 bp of the *SGS1* coding sequence. The possibility of downstream readthrough producing a Leu2:Sgs1 fusion protein can be eliminated due to the presence of intervening termination codons. The truncated Sgs1 polypeptide that might be expressed in strains carrying a deletion of *SGS1* would be expected to lack all of the residues conserved in RecQ, including the seven helicase motifs, as well as the domain of interaction between Sgs1p and topoisom-

TABLE 1

Strains

Strain name	Markers
Y55 congenic background	
PW10	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H*
PW10ΔSGS1	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H sgs1::LEU2*
PW10ΔRAD52	*his4-C leu2-R MATa-URA3 MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H rad52::LEU2*
PW10ΔSGS1ΔRAD52	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H sgs1::LEU2 rad52::LEU2*
PW20	*HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1*
PW20ΔSGS1	*HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1 sgs1::LEU2*
PW80	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H* *HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1*
PW80ΔSGS1	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H* *HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1* *sgs1::LEU2* *sgs1::LEU2*
PW80ΔRAD52	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H* *HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1* *rad52::LEU2* *rad52::LEU2*
PW80ΔSGS1ΔRAD52	*his4-C leu2-R MATa-URA3-MATa met13-4 cyh2^R TRP5 lys2-d ura3-1 CAN1 ade1-1 trp1-H* *HIS4 leu2-K MATα-URA3-MATα met13-2 cyh2^R trp5-1 lys2-c ura3-1 can1^R ade1-1 TRP1* *sgs1::LEU2 rad52::LEU2* *sgs1::LEU2 rad52::LEU2*
Y55 background	
RHB2180	*his4-R leu2-R MATa THR4 met13-4 LYS2 ura3-N CAN1 ade1-1* *HIS4 LEU2 MATα thr4-A MET13 lys2-D ura3-N can1^R ADE1*
Y55-D106	*his4-R leu2Δ MATa thr4-A met13-4 ura3-N can1^R ade1-1* *HIS4 leu2Δ MATα THR4 met13-4 ura3-N CAN1 ADE1* *TRP1 sgs1::LEU2* *trp1-B sgs1::LEU2*
Y55-D107	*HIS4 leu2Δ MATa met13-4 LYS2 ura3-N* *his4-R leu2Δ MATα met13-4 lys2-C ura3-N* *trp1-B sgs1::LEU2* *TRP1 sgs1::LEU2*
Y55-D108	*leu2Δ MATa MET13/14 CYH2 LYS9 ura3-N* *leu2Δ MATα met13 or 14 cyh2R lys9-1 ura3-N* *TRP1 ade8-1 sgs1::LEU2* *trp1::URA3 ADE8 sgs1::LEU2*
Y55-D109	*leu2Δ MATa MET13/14 CYH2 LYS9 ura3-N* *leu2Δ MATα met13 or 14 cyh2R lys9-1 ura3-N* *TRP1 ade8-1* *trp1::URA3 ADE8*
YP1 background	
EJL605-2D	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2*
PW100	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 sgs1::LEU2*
EJL610-1C	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 rad1::LEU2*
EJL605-3B	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 rad52::LEU2*
PW120	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 sgs1::LEU2 rad1::LEU2*
EJL609-2B	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 sgs1::LEU2 rad52::LEU2*
EJL610-2A	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 rad1::LEU2 rad52::LEU2*
EJL611-13A	*his4-R leu2Δ MATa-URA3-MATa ura3-52 ade2-101 lys2 sgs1::LEU2 rad1::LEU2 rad52::LEU2*
EJL601-6A	*HIS4 leu2Δ MATα ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ XV R*
EJL601-5D	*HIS4 leu2Δ MATα ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ XV R sgs1::LEU2*
EJL612-3C	*his4-R leu2Δ MATa ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ XV R top3::LEU2*
EJL613-1D	*his4-R leu2Δ MATa ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ XV R sgs1::LEU2 top3::LEU2*
EJL602-7C	*HIS4 leu2Δ MATa ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ IX L*
EJL602-6C	*HIS4 leu2Δ MATa ura3-52 ade2-101 lys2 Y'::URA3 SUP11 @ IX L sgs1::LEU2*

erase II. Further evidence that the *sgs1*Δ strains lack functional Sgs1p comes from the observation that the phenotype of an *sgs1*Δ strain is the same as that of *sgs1* point mutants, which are all considered to be effective nulls (GANGLOFF *et al.* 1994).

Mutation of *SGS1* causes a mitotic hyperrecombination phenotype: Because of the involvement of RecQ in genetic recombination in *E. coli*, and the apparent hyperrecombination phenotype of Bloom's syndrome cell lines, the effect of deleting the *SGS1* gene on the frequency of different forms of mitotic recombination in *S. cerevisiae* was analyzed. The structures of the various substrates used in the recombination assays are displayed in Figure 2.

Intrachromosomal recombination: To analyze intrachromosomal "excision" recombination, the rate of loss of a genetic marker was quantified in three assays using the median method of LEA and COULSON (1949). In the first assay, the rate of loss of the *URA3* gene (monitored by the acquisition of resistance to 5-FOA), integrated between two homologous *MAT* loci, was quantified in strains PW10 and PW20 (see Figure 2A). Table 2 shows that deletion of *SGS1* caused a 3.3-fold increase in the rate of loss of *URA3* at the *MAT* locus in PW10, and a 12-fold increased rate of loss in PW20 (both increases being significant). The difference in rate reflects an apparent general difference in recombination rates between *MAT***a** and *MAT*α cells. While deletion of *RAD52* reduced the background rate of *URA3* loss by approximately fourfold, a *sgs1*Δ *rad52*Δ double mutant showed a level of marker loss comparable to that of a *sgs1*Δ mutant, and nearly 10-fold above that of the *rad52*Δ mutant (Table 2). We conclude, therefore, that the rate of loss of *URA3* at *MAT* is elevated in a *sgs1*Δ strain and that this elevated frequency of marker loss occurs predominantly via a *RAD52*-independent pathway.

To determine whether the increased rate of loss in an *sgs1*Δ mutant was seen with a second marker located at the same locus in a different genetic background, the rate of loss of the *CYH2* gene from the *MAT-MAT* interval was quantified by measurement of the induction of cycloheximide resistance in the YP1 strains (see Figure 2B). In this case, deletion of *SGS1* caused a smaller, but reproducible, increase in the rate of marker loss (Table 2). This difference between recombination rates in the experiments described in Table 2 could be due to the fact that *MAT***a** cells have a lower rate of recombination in general, that the strain backgrounds are different, or that the assay structure is different between the strains. In the YP1 genetic background, the effects on recombination frequencies of mutations in both the *RAD1* and *RAD52* genes were also determined. Table 2 shows that while mutation of either *RAD1* or *RAD52* had a small negative effect on the frequency of *CYH2* excision from the *MAT* interval, deletion of these two genes together produced a 20-fold reduction in the rate of marker loss. However, the *sgs1*Δ *rad1*Δ *rad52*Δ triple mutant showed a rate of *CYH2* gene loss fourfold above that of a *rad1*Δ *rad52*Δ double mutant (a significant increase), indicating that at least a proportion of the elevated recombination associated with mutation of *SGS1* is independent of both *RAD1* and *RAD52*. Since the strain used in this study had *URA3* as well as *CYH2* between the *MAT* loci, it was possible to monitor gene conversions between the $cyh2^R$ allele on chromosome *VII* and the *CYH2* gene in the *MAT* interval (genetically scored as cyh^R Ura^+). There was no effect of *sgs1*Δ on the rate of these gene conversion events (rates of 1.18×10^{-7} in SGS1 *vs.* 1.54×10^{-7} in *sgs1*Δ).

In a third analysis of possible intrachromosomal recombination, the rate of loss of the *URA3* gene from a subtelomeric site was determined (see Figure 2C). In previous studies of Y′ stability, it was found that loss of the *URA3* could occur either by intrachromosomal excision of the whole Y′ element via recombination involving the flanking $TG_{1\text{-}3}$ repeats, or by ectopic recombination with unmarked Y′ elements at other chromosomal locations (LOUIS and HABER 1990). In previous studies it was found that excision recombination represented at most 10% of the *URA3* loss events (LOUIS and HABER 1990; LOUIS *et al.* 1994). Deletion of *SGS1* caused a 10-fold increase in the rate of *URA3* gene loss from the end of chromosome *XV* or the end of chromosome *IX*. It should be noted that excision using flanking $TG_{1\text{-}3}$ sequences was not possible from the end of chromosome *IX*. The similar increase in *URA3* gene loss from both the right end of chromosome *XV* and the left end of chromosome *IX* (Table 3) thus indicates that ectopic interactions with other Y′ elements (rather than excision) was a major cause of *URA3* gene loss in *sgs1*Δ strains. Thus, the stimulation of recombination in *sgs1*Δ strains is not specific for the *MAT* locus and involves both intrachromosomal excision and interchromosomal ectopic events.

Interchromosomal mitotic recombination between heteroalleles: Strains PW10 and PW20 (Table 1) were constructed such that recombination between heteroalleles in a diploid (*i.e.*, PWD80 and its corresponding homozygous isogenic *sgs1*Δ mutant, PWD80ΔSGS1) could be detected via a restoration of lysine and methionine prototrophy (BORTS *et al.* 1986). A consistent stimulation in the average recombination rate (an average 14-fold for the *met13* locus and 3.2-fold for the *lys2* locus) was observed for diploid PWD80ΔSGS1, when compared to the isogenic control diploid PWD80 (Table 4). This level of hyperrecombination was also observed in a second PWD80ΔSGS1 diploid strain made by mating independently constructed PW20ΔSGS1 and PW10ΔSGS1 haploids (Table 4). This increase in prototroph formation was not due to reversions of the *met13* and *lys2* alleles as there was no increase in prototroph

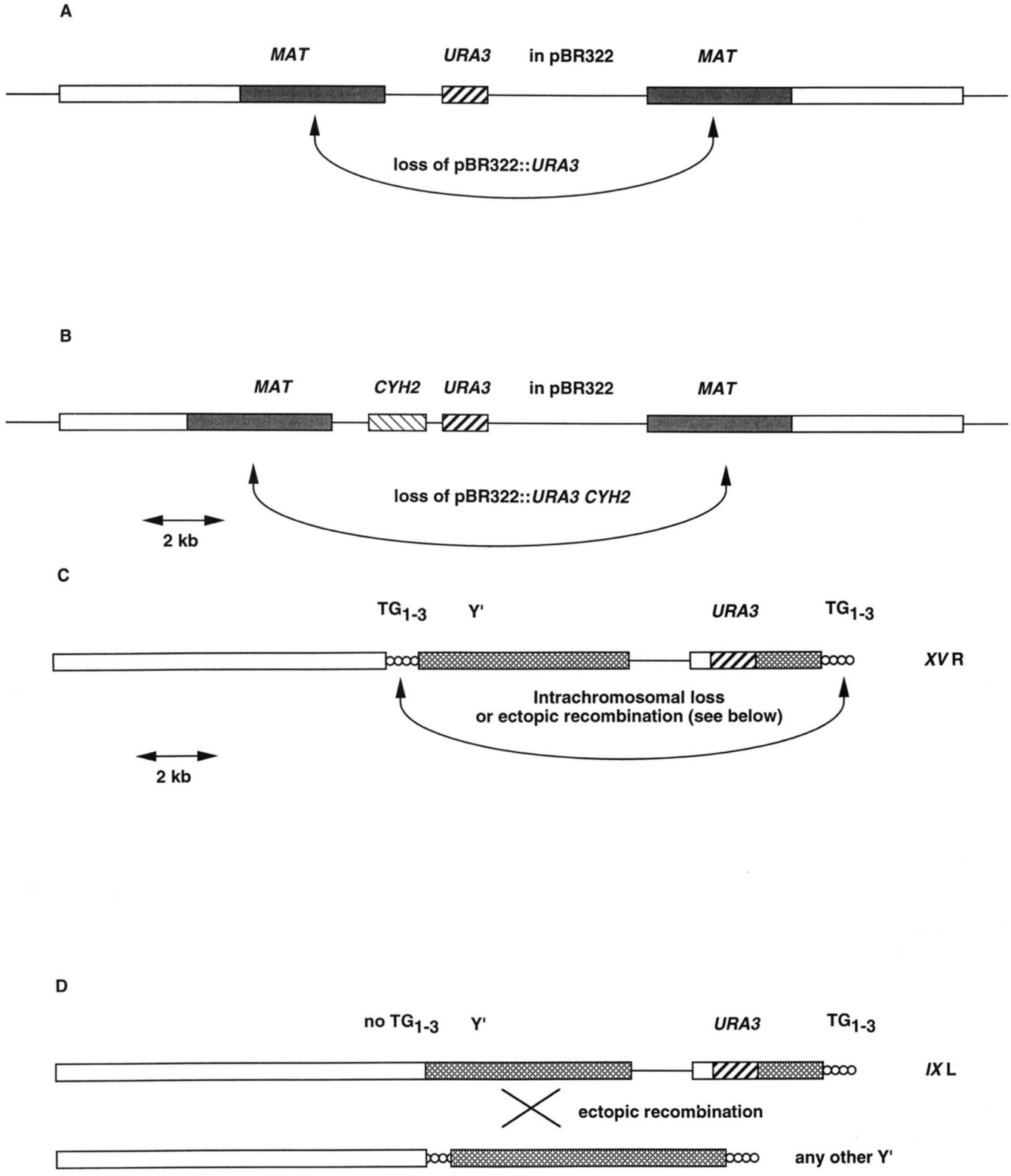

FIGURE 2.—A schematic representation of the structure of the recombination assays used is shown. (A) The *MAT-URA3-MAT* interval in which intrachromosomal loss of the intervening sequences was monitored. (B) The same interval with the addition of the *CYH2* gene. (C) The structure of the marked Y′ at the right end of chromosome *XV* in which both intrachromosomal loss and interchromosomal ectopic recombination can occur. (D) The structure of the marked Y′ at the left end of chromosome *IX* in which only interchromosomal ectopic recombination can lead to marker loss.

TABLE 2

Loss of markers at the *MAT* interval

Strain	Genotype	Rate of FOA^R ($\times 10^6$)
PW10	wt	6.0
PW10ΔSGS1	*sgs1*Δ	19.8 *[a](>wt)
PW10ΔRAD52	*rad52*Δ	1.4
PW10ΔSGS1ΔRAD52	*sgs1*Δ *rad52*Δ	13.0 *(>*rad52*Δ)
PW20	wt	0.8
PW20ΔSGS1	*sgs1*Δ	10.0 *(>wt)

Strain	Genotype	Rate of Cyh^R ($\times 10^6$)
EJL605-2D	wt	13.4
PW100	*sgs1*Δ	22.3
EJL610-1C	*rad1*Δ	5.2
PW120	*sgs1*Δ *rad1*Δ	14.0 *(>*rad1*Δ)
EJL605-3B	*rad52*Δ	8.2
EJL609-2B	*sgs1*Δ *rad52*Δ	8.6
EJL610-2A	*rad1*Δ *rad52*Δ	0.8 *(<wt, *rad1*Δ and *rad52*Δ)
EJL611-13A	*sgs1*Δ *rad1*Δ *rad52*Δ	3.2 *(>*rad1*Δ*rad52*Δ)

[a] Significantly different from the strain indicated (*$P < 0.05$; see MATERIALS AND METHODS). wt, wild type.

formation in any of the haploid *sgs1*Δ strains (data not shown).

As expected, mutation of *RAD52* in PWD80 diminished the level of heteroallelic recombination at *MET13* and *LYS2* to below the level of detection of our assay. In contrast, while deletion of *RAD52* in a *sgs1*Δ strain reduced the rate of interchromosomal recombination by ~20-fold compared to wild-type PWD80, a measurable level of residual recombination was evident in the *sgs1 rad52* double mutant (Table 4), indicating that at least a proportion of the *SGS1*-dependent interchromosomal hyperrecombination was *RAD52*-independent.

Sporulation of homozygous *sgs1*Δ diploids results in reduced meiotic viability, but no apparent associated recombination defect: We have reported previously that *sgs1*Δ strains show reduced tetrad formation and spore viability compared to isogenic controls. To assess whether this inviability was associated with any recombination defects (in addition to the previously described segregation defects), several experiments were performed.

TABLE 3

Rate of Y′::*URA3* loss

Strain	Genotype	Rate of FOA^R ($\times 10^6$)
EJL601-6A	wt (*XV* R)[a]	3.6
EJL601-5D	*sgs1*Δ (*XV* R)	36.5 *[b](>wt)
EJL612-3C	*top3*Δ (*XV* R)	2.8
EJL613-1D	*sgs1*Δ*top3*Δ(*XV* R)	10.0
EJL602-7C	wt (*IX* L)	0.6
EJL602-6C	*sgs1*Δ (*IX* L)	5.4 *(>wt)

[a] The chromosome (*XV* or *IX*) and the end (left or right) at which the Y′::*URA3* is integrated is indicated. wt, wild type.

[b] Significantly different from the strain indicated (*$P < 0.05$; see MATERIALS AND METHODS).

Random spore analysis was performed to assess the rates of heteroallelic recombination at the *MET13* and *LYS2* loci. The frequency of generation of Met$^+$ prototrophy in *SGS1* strains was 0.59% of spores, translating to a rate of 0.0236 prototrophs per meiosis, which agrees with previous studies using these heteroalleles (BORTS *et al.* 1986). From an average of seven experiments with the *sgs1*Δ strain, a Met$^+$ frequency of 0.9% of spores was obtained. This marginally elevated rate is not significant. Similarly, the frequencies of Lys$^+$ prototrophs were 0.22% and 0.18% in *SGS1* and *sgs1*Δ strains, respectively.

Tetrads from diploids Y55-D106, Y55-D107 and RHB2180 were dissected to assess the effects of *sgs1*Δ on crossing over in the *HIS4* to *CEN* and *CEN* to *MAT* intervals on chromosome *III*. For the *HIS4* to *CEN* interval, there were 42/82 ditypes (51%) in the combined *sgs1*Δ strains *vs.* 121/241 (50%) in the *SGS1* strain. Similarly, there was no difference in crossing over in

TABLE 4

Mitotic rate of heteroallele recombination

Strain	Genotype	Rate of Met$^+$ ($\times 10^7$)	Rate of Lys$^+$ ($\times 10^7$)
pWD80	wt	5.8	24.0
PWD80ΔSGS1-A	*sgs1*Δ	76.0*[a]	72.0*
PWD80ΔSGS1-B	*sgs1*Δ	83.0*	88.0*
PWD80ΔRAD52	*rad52*Δ	<0.1[b]	<0.1[b]
PWD80ΔSGS1ΔRAD52	*sgs1*Δ *rad52*Δ	0.5	1.1

wt, wild type.

[a] Significantly different from the strain indicated (*$P < 0.05$; see MATERIALS AND METHODS).

[b] The assay should have detected a rate of 0.1×10^7 given the number of cells plated.

the *CEN* to *MAT* interval, where there were 61/92 (66%) and 160/257 (62%) ditypes in the *sgs1*Δ and *SGS1* strains, respectively.

Strains Y55-D108 and Y55-D109, which are isogenic to the strains used to show meiotic missegregation in *sgs1*Δ strains (WATT *et al.* 1995), were constructed with a distal auxotrophic marker to assess the recombination status of missegregated chromosomes. Random spores of these strains showed the same increase in disome production as previously described (WATT *et al.* 1995), but no difference in recombination status of the disomes. In both the *sgs1*Δ and the *SGS1* strains, the disomes produced were homozygous for the distal auxotrophic *ade8* marker in ~25% of the cases (32/141 or 23% and 72/360 or 20% for the *sgs1*Δ and *SGS1* strains, respectively), which is the expected frequency for random association of recombination and missegregation of chromosome *IV*.

***SGS1* and *TOP3* have no apparent role in the maintenance of telomere integrity:** Topoisomerase III has been shown previously to form a complex with Sgs1p in *S. cerevisiae*, indicating that these two proteins probably act in concert. Consistent with there being a functional association between Sgs1p and topoisomerase III, a mutant allele of SGS1 has been identified that acts as a suppressor of the slow-growth phenotype of a *top3*Δ mutant (GANGLOFF *et al.* 1994). Based on our finding that *SGS1* influences recombination within subtelomeric sequences, together with the previous observation of KIM *et al.* (1995) that telomere structure and stability are altered in *top3* mutants, the effect on telomere integrity of deletions in the *SGS1* and *TOP3* genes was analyzed. To achieve this, *sgs1*Δ and *top3*Δ derivatives as well as a *sgs1*Δ *top3*Δ double mutant were constructed in the YP1 background (Table 1). In agreement with the data of GANGLOFF *et al.* (1994), in which a point mutant of *SGS1* was analyzed, deletion of *SGS1* suppressed the slow-growth phenotype of a *top3*Δ strain (data not shown). However, in contrast to the stimulatory effects of a *TOP3* gene deletion on the frequency of excision recombination within repetitive δ elements and rDNA (WALLIS *et al.* 1989), no evidence was obtained for an effect of deleting *TOP3* on the rate of loss of *URA3* from the right end of chromosome *XV* telomeric locus (Table 3). Moreover, the integrity of telomeric sequences appeared to be unaltered by deletion of either *TOP3* alone, or *TOP3* and *SGS1* together. Figure 3 shows that deletion of these genes did not influence either the number of copies of subtelomeric Y′ elements, or the length of the $TG_{1\text{-}3}$ tracts, even when these strains were grown for over 100 generations.

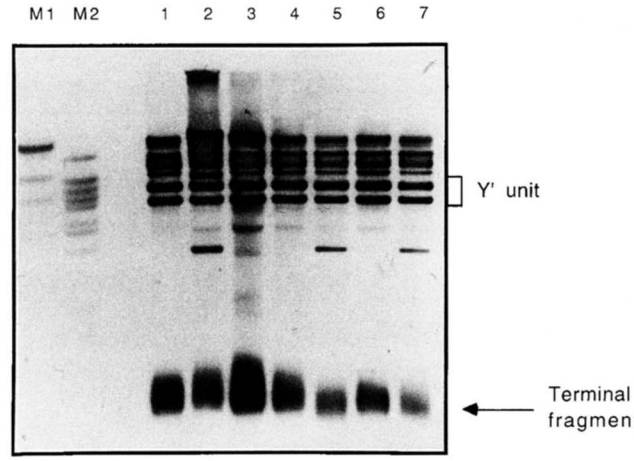

FIGURE 3.—The structure of the telomere tracts and Y′ elements in *sgs1*Δ and *top3*Δ strains as assessed by Southern blotting analysis. *Xho*I fragments of genomic DNA from wild-type (lanes 3 and 6), *sgs1*Δ (lanes 1 and 4), *top3*Δ (lanes 2 and 5) and *sgs1*Δ *top3*Δ (lane 7) strains were separated on an 0.8% agarose gel. Markers in lanes M1 and M2 are lambda DNA digested with *Hin*dIII and *Bst*EII, respectively. The DNA in lanes 1–3 and 7 was isolated after ~20 generations, while the DNA in lanes 4–6 was isolated only after culture propagation for over 100 generations. pEL30, which contains both ends of Y′ elements and ~150 bp of $TG_{1\text{-}3}$ sequences, was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) and used as a probe according to the manufacturer's intstructions. The typical 1.1- to 1.4-kb diffuse terminal telomeric *Xho*I fragments are indicated on the right, as are the 5.4 and 6.7 kb Y′ homologous fragments arising from tandem Y′ tracts. The *top3*Δ disruption contains vector homology resulting in the extra 3.7-kb fragment in lanes 2, 5 and 7.

DISCUSSION

Using a two-hybrid cloning strategy, we have previously reported the isolation of a gene from *S. cerevisiae*, designated *SGS1*, encoding a member of the RecQ family of DNA helicases (WATT *et al.* 1995). More recent data indicate that the closest structural homologues to *SGS1* are the *BLM* and *WRN* genes, which are defective in individuals with Bloom's syndrome (ELLIS *et al.* 1995; reviewed in WATT and HICKSON 1996) and Werner's syndrome (YU *et al.* 1996), respectively. We have shown here that strains lacking a functional *SGS1* gene exhibit abnormally high rates of mitotic recombination.

The RecQ protein is not required for genetic recombination in a wild-type *E. coli* background. However, a *recQ*$^+$ genotype is required for recombination in certain mutant backgrounds. For example, in *recBC sbcA*, or *recBC sbcBC* backgrounds, RecQ is required for intramolecular plasmid recombination and conjugation mediated recombination (NAKAYAMA *et al.* 1984, 1985; LUISI-DELUCA *et al.* 1989). These results and others suggest that RecQ participates in the RecF recombination pathway (reviewed in WEST 1992, 1994). The RecQ helicase may cooperate with the RecJ nuclease in generating the single-stranded DNA necessary for initiating strand transfer during genetic recombination and/or repair processes (KUSANO *et al.* 1994).

Deletion of *SGS1* causes mitotic hyperrecombination: The phenotype of the yeast strain lacking functional Sgs1 protein is clearly distinct from that of an *E. coli recQ* mutant. Far from being deficient in genetic

recombination, *sgs1* mutants display a hyperrecombination phenotype during mitotic growth. In this respect, the phenotype of a *sgs1* strain closely resembles that of a Bloom's syndrome cell line, providing evidence that Sgs1p and BLM are functional as well as structural homologues. The hyperrecombination observed in *sgs1*Δ strains is manifested as an increase in both intra- and interchromosomal homologous recombination at more than one locus. We also confirmed previous data (GANGLOFF *et al.* 1994) showing that *sgs1* strains have an elevated rate of marker loss from the rDNA locus (data not shown). The observed increase in intramolecular excision at the *MAT* locus indicates that, unlike topoisomerase II, with which Sgs1p interacts, the action of Sgs1 in "suppressing" excessive excision recombination is not confined to the highly repetitive rDNA gene cluster.

A proportion of the hyperrecombination in *sgs1* strains is independent of the *RAD52* and *RAD1* pathways: Mutation of *RAD52* eliminates most meiotic recombination (GAME *et al.* 1980; BORTS *et al.* 1986) and results in a very substantial reduction in the frequency of mitotic heteroallelic homologous recombination at several loci (reviewed in PETES *et al.* 1991). However, a minor fraction of heteroallelic recombination persists in *rad52* strains, suggesting that at least one *RAD52*-independent recombination pathway exists in *S. cerevisiae.* Recent evidence suggests that both gene conversions and reciprocal crossover events at tandem duplications are generally *RAD52*-dependent. However, a nonconservative, *RAD52*-independent pathway for intrachromosomal excision recombination also exists, and the frequency of many such excision events is only partially reduced in *rad52* mutants (THOMAS and ROTHSTEIN 1989; reviewed in KLEIN 1995). For example, the stimulation in excision frequency at the rDNA locus in many hyperrecombination mutants, such as *rrm3* and *top1* (CHRISTMAN *et al.* 1988; KEIL and MCWILLIAMS 1993), is not eliminated by a mutation in *RAD52* (KLEIN 1995). Many of these *RAD52*-independent events require a functional *RAD1* gene, and Rad1p is generally thought to participate in single-stranded DNA annealing processes. Elimination of both *RAD1* and *RAD52* generally gives a synergistic reduction in the frequency of intrachromosomal excision recombination (LIEFSHITZ *et al.* 1995), as was found in our study.

Our data indicate that deletion of *SGS1* causes a hyperrecombination phenotype that is at least partially independent of *RAD52* for heteroallelic recombination, and of both *RAD1* and *RAD52* for intrachromosomal recombination. This suggests that Sgs1p is unlikely to act solely as a participant in these two well characterized recombination pathways in *S. cerevisiae.* The lack of sensitivity to ionizing radiation in *sgs1*Δ mutants (our unpublished data) indicates that recombinational repair of DNA double-strand breaks is still functional in the absence of Sgs1p. Further work is needed to ascertain whether the Sgs1 protein is involved in removal of other classes of DNA lesion requiring recombinational repair. However, unlike *E. coli recQ* mutants, *sgs1*Δ strains are not hypersensitive to UV light (our unpublished data).

Deletion of *SGS1* has no apparent effect on meiotic recombination: A significant level of spore death was observed with *sgs1*Δ diploids in tetrad dissection experiments. This is consistent with the observation that meiotic missegregation (both meiosis I nondisjunction and precocious sister segregation were measured) is greatly elevated in *sgs1*Δ strains (WATT *et al.* 1995). However, this nonviability and increased aneuploidy was not accompanied by any measurable change of rate in recombination among the viable spores both at heteroalleles and in standard genetic intervals. Furthermore, the aneuploids produced in *sgs1*Δ strains show no difference in recombination frequency compared to *SGS1* strains. Clearly the meiotic recombination analyses presented here cannot take into account the levels of recombination in nonviable progeny. It is a formal possibility, therefore, that an abnormal level of recombination was responsible for some of the reduction in meiotic viability.

***SGS1* and *TOP3* have no apparent role in telomere maintenance:** We have shown that loss of *URA3* from a subtelomeric site occurs at an elevated frequency in *sgs1* strains, although the structural integrity (loss of Y′ elements and $TG_{1\text{-}3}$ tract length) of the telomeres is not obviously influenced by loss of Sgs1p function. In the light of a previous study on the role of topoisomerase III in the maintenance of telomere integrity (KIM *et al.* 1995), we were surprised to find no evidence for an elevated rate of Y′ element loss or $TG_{1\text{-}3}$ tract shortening in either a *top3*Δ strain or a *top3*Δ *sgs1*Δ double mutant. The explanation for these conflicting results remains elusive, although one possibility is that the deletion of *TOP3* made by KIM *et al.* (1995) caused an inadvertent inactivation of the adjacent, divergently transcribed, *EST1* gene. Mutations in *EST1* lead to Y′ element loss and $TG_{1\text{-}3}$ tract shortening (LUNDBLAD *et al.* 1989).

Is the association of Sgs1p and topoisomerases of functional significance in genetic recombination? Topoisomerase II may have evolved a means of recruiting one of the nuclear DNA helicases (*i.e.*, Sgs1p) to a site where it could be used more efficiently to facilitate the resolution of newly replicated chromosomes (described in WATT *et al.* 1995). It is also clear from studies in *E. coli* that a type II topoisomerase is needed to remove knots and catenanes generated during recombination processes (BLISKA and COZZARELLI 1987; ADAMS *et al.* 1992). Thus, an interaction between the Sgs1 and topoisomerase II proteins might be an integral part of the mechanism by which the respective roles of these two proteins are coordinated during both late-stage replication and mitotic recombination. However, it is not clear if any connection exists between the stimulation of mitotic intrachromosomal excision observed in *sgs1*Δ mutants, and the hyperrecombination seen in *top2* mu-

tants. Mutations in either *TOP1* or *TOP2* (and synergistically in the double mutant) cause hyperrecombination only at the *rDNA* locus (CHRISTMAN *et al.* 1988; KIM and WANG 1989). It has been postulated that this stimulation of recombination is due to a build-up of DNA supercoils as a result of the unusually high rate of *rDNA* gene transcription. Sgs1 protein might participate in the suppression of *rDNA* excision events and/or act to facilitate the reintegration of *rDNA* rings. If the association of the Sgs1 protein and topoisomerase II proteins was specifically required for this purpose, it is difficult to explain why a similar effect on hyperrecombination at the telomeric, *MAT* and the *rDNA* loci was observed in *sgs1*Δ strains, particularly since the telomeric and *MAT* loci might not be expected to be as supercoiled as the highly transcribed *rDNA* locus.

Recently, GANGLOFF *et al.* (1994) reported that the slow growth phenotype of *top3* mutants is suppressed by a mutation in *SGS1*. We have confirmed that this suppression phenomenon results from loss of function of *SGS1* and not from the expression of an abnormal Sgs1p encoded by the *sgs1* allele reported by GANGLOFF *et al.* (1994). The function of topoisomerase III remains unclear, although it would appear to perform an important cellular role because *top3* strains grow more slowly than their wild-type counterparts. In the absence of the topoisomerase III (an enzyme with very poor DNA relaxation activity), recombination of δ repeat sequences (WALLIS *et al.* 1989), *rDNA* repeats (GANGLOFF *et al.* 1994) and *SAM* repeats (BAILIS *et al.* 1992) is stimulated. It is possible that the similar phenotypes of *top3* and *sgs1* mutants could be due to a common, perhaps concerted, mechanism of action of these gene products. The pleiotropic effects of a loss of Sgs1 helicase on interchromosomal as well as intrachromosomal recombination could, therefore, result from a failure to unwind inappropriately paired DNA strands. Such a resolution function could in principle be coordinated with a topoisomerase acting on plectonemically intertwined DNA, as proposed by WANG *et al.* (1990).

Possible roles of *SGS1* and the *BLM* and *WRN* proteins: Based on the phenotype of *sgs1* mutants and the known defects in the synthesis of full-length replicons in Bloom's syndrome cell lines, we have postulated elsewhere that the elevated frequencies of both recombination and chromosome missegregation in *sgs1*Δ strains could arise via one of two mechanisms (WATT and HICKSON 1996). Briefly, one mechanism is that Sgs1p is required during late stage replication to unwind topologically constrained domains (*i.e.*, converging replication forks), allowing completion of replication, before decatenation by topo II. In the absence of Sgs1p, these incompletely replicated sister chromatids would be broken during an attempt to segregate sister chromatids that remain intertwined. This might be the mechanism by which recombinogenic lesions are generated. A second possible mechanism is that blocked or collapsed replication forks (BIERNE and MICHEL 1994) might require recombination functions to restore replication elongation, and that Sgs1p participates in this recombination "repair" pathway. In the absence of Sgs1p, replication fork breakage might lead to persistence of recombinogenic strand breaks at replication forks and to a decrease in cell viability.

Although WRN cell lines are not known to show a dramatic elevation in homologous recombination, WRN cells do have a shorter replication life (associated with abnormal telomere maintenance), a decreased efficiency of ligation and a possible abnormal replication (see YU *et al.* 1996 and references therein). In humans, it appears that the BLM and WRN proteins have evolved to perform distinct but possibly overlapping functions, while in yeast Sgs1p, the single RecQ family member in this organism, presumably performs a wide range of functions. Based upon sequence comparisons and phenotype comparisons of mutants, it appears that Sgs1p is more functionally homologous to BLM than to WRN, yet the functions of WRN could still be related in mechanism if not in phenotypic outcome.

In summary, we have identified a role for the *S. cerevisiae SGS1* gene in the maintenance of genome stability. The challenge now is to understand the biochemical basis for the defect in genetic recombination in *sgs1* strains and to ascertain whether abnormal recombination is responsible for the observed chromosome missegregation phenotype of these mutants.

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