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Sgs1 Helicase Activity Is Required for Mitotic but Apparently Not for Meiotic Functions

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The *SGS1* gene of *Saccharomyces cerevisiae* is a homologue for the Bloom's syndrome and Werner's syndrome genes. The disruption of the *SGS1* gene resulted in very poor sporulation, and the majority of the cells were arrested at the mononucleated stage. The recombination frequency measured by a return-to-growth assay was reduced considerably in *sgs1* disruptants. However, double-strand break formation, which is a key event in the initiation of meiotic DNA recombination, occurred; crossover and noncrossover products were observed in the disruptants, although the amounts of these products were slightly decreased compared with those in wild-type cells. The spores produced by *sgs1* disruptants showed relatively high viability. The *sgs1 spo13* double disruptants sporulated poorly, like the *sgs1* disruptants, but spore viability was reduced much more than with either *sgs1* or *spo13* single disruptants. Disruption of the *RED1* or *RAD17* gene partially alleviated the poor-sporulation phenotype of *sgs1* disruptants, indicating that portions of the population of *sgs1* disruptants are blocked by the meiotic checkpoint. The poor sporulation of *sgs1* disruptants was complemented with a mutated *SGS1* gene encoding a protein lacking DNA helicase activity; however, the mutated gene could suppress neither the sensitivity of *sgs1* disruptants to methyl methanesulfonate and hydroxyurea nor the mitotic hyperrecombination phenotype of *sgs1* disruptants.

Proteins having DNA helicase activity play important roles in many processes involving DNA, such as replication, repair, and recombination. The product of the *Escherichia coli recQ* gene, which has DNA helicase activity, is a member of the RecF pathway of recombination. The *recF* mutants lack conjugal recombination proficiency and UV resistance in the background of *recBCD* (lacking active endonuclease V) and *sbcBC* (lacking active exonuclease I), and *recQ* deletion mutants in the background of *recBC sbcBC* display UV and methyl methanesulfonate (MMS) sensitivity (22, 30).

We (38) and Puranam and Blackshear (32) cloned cDNAs encoding a RecQ homologue of human cells, DNA helicase Q1 and RECQL, respectively. Since these are the same gene, we tentatively designated this gene *RECQL1.* We also cloned a gene of *Saccharomyces cerevisiae* encoding a protein having DNA helicase motifs with high homology to those of *E. coli* RecQ and human ATPase *RECQL1*. This gene soon was found to be identical to the *SGS1* (slow growth suppressor 1) gene.

A mutant allele of the *SGS1* gene was identified as a suppressor of the slow-growth phenotype of *top3* mutants (11). Two-hybrid experiments indicated that the yeast Sgs1 protein interacts with DNA topoisomerase III (Top3) (11) as well as DNA topoisomerase II (50). The protein encoded by the *SGS1* gene has seven conserved helicase motifs, and Sgs1 was shown to actually have DNA helicase activity (3, 23). Deletion mutants of the *SGS1* gene showed a reduction in the fidelity of chromosome segregation during mitosis and meiosis (50, 51); mitotic hyperrecombination phenotypes in interchromosomal homologous recombination, intrachromosomal excisional recombination, ectopic recombination (51), unequal sister chromatid recombination (31), and illegitimate recombination (53); and premature aging (44). The *sgs1* mutants were shown to be moderately sensitive to MMS (10) and hydroxyurea (HU) (53) but not to ionizing radiation or UV light (51).

Four human genes encoding a RecQ homologue have been identified in addition to *RECQL1*. These are the Bloom's syndrome (*BLM*) gene (8), the Werner's syndrome (*WRN*) gene (54), *RECQL4* (19, 20), and *RECQL5* (19). The representative clinical manifestations of Bloom's syndrome (BS) are cancer predisposition, immunodeficiency, and male infertility (13, 16). In BS cells, the interchanges between homologous chromosomes are increased and an abnormally large number of sister chromatid exchanges are present (13). Werner's syndrome (WS) patients prematurely develop a variety of major age-related diseases, such as arteriosclerosis, malignant neoplasms, melituria, and cataracts (9). The cells derived from WS patients show chromosome instability and a shorter life span in cultures (28). However, it remains unclear how the dysfunction of the gene products is related to the observed phenotypes of cells derived from these patients.

To date, a number of RecQ homologues have been reported from prokaryotes, such as *Bacillus subtilis* (L47648 in GenBank) and *Haemophilus influenzae* (HI32756 in GenBank); eukaryotes, such as *S. pombe* (45) and *S. cerevisiae*; and higher eukaryotes, including humans. Although significant homology is present within the consensus helicase domains, these RecQ

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TABLE 1. Experimental strains of *S. cerevisiae*

Strain	Genotype	Reference or source
W303-1A	*MAT***a** *ura3-1 leu2-3,112 trp1-1 his3-11 ade2^{oc} can1-100 Gal^{+}*	Thomas and Rothstein (48)
WQ701	*MAT***a**/*MAT*α *W303-1A but sgs1::URA3*	This study
YPH499	*MAT***a**/*MAT*α *ade2-100^{oc} leu2Δ his3Δ200 lys2-801^{am} ura3-52 trp1Δ63 Gal^{+}*	Sikorski and Hieter (43)
YQ401	*MAT***a**/*MAT*α YPH499 but *sgs1::URA3*	This study
MR966	*MAT***a**/*MAT*α *ura3-52 leu2-3,112 trp1-289 his1-7*	Resnick et al. (33)
MR93-28C	*MAT***a**/*MAT*α *ura3-52 leu2-3,112 trp1-289 his1-1*	Resnick et al. (33)
MR101	*MAT***a**/*MAT*α MR966/MR93-28c	This study
MR202	*MAT***a**/*MAT*α MR966 but *sgs1::URA3*/MR93-28c but *sgs1::LEU2*	This study
MR301 (ds1)	*MAT***a**/*MAT*α MR966 but *sgs1Δ::AUR*/MR93-28c but *sgs1Δ::AUR*	This study
ds13	*MAT***a**/*MAT*α MR966 but *spo13::URA3*/MR93-28c *but spo13::URA3*	This study
ds1m11	*MAT***a**/*MAT*α MR966 but *sgs1Δ::AUR mre11::URA3*/MR93-28c *but sgs1Δ::AUR mre11::URA3*	This study
ds1s13	*MAT***a**/*MAT*α MR966 but *sgs1Δ::AUR spo13::URA3*/MR93-28c *but sgs1Δ::AUR spo13::URA3*	This study
ds13m11	*MAT***a**/*MAT*α MR966 but *mre11::URA3 spo13::hisG*/MR93-28c but *mre11::URA3 spo13::hisG*	This study
dr1	*MAT***a**/*MAT*α MR966 but *red1::URA3*/MR93-28c *but red1::URA3*	This study
dr17	*MAT***a**/*MAT*α MR966 but *rad17::LEU2*/MR93-28c *but rad17::LEU2*	This study
ds1r1	*MAT***a**/*MAT*α MR966 but *sgs1Δ::AUR red1*::URA3/MR93-28c but *sgs1Δ::AUR red1::URA3*	This study
ds1r17	*MAT***a**/*MAT*α MR966 but *sgs1Δ::AUR rad17::LEU2*/MR93-28c but *sgs1Δ::AUR rad17::LEU2*	This study
NKY1303	*MAT***a**/*MAT*α *lys2 ho::LYS2 ura3 leu2::hisG arg4-bgl his4B::LEU2*	Storlazzi et al. (46)
NKY1543	*MAT***a**/*MAT*α *lys1 ho::hisG ura3 leu2::hisG arg4-nsp his4X::LEU2* (*Bam*HI)-*URA3*	Storlazzi et al. (46)
RKH112	*MAT***a**/*MAT*α NKY1303 but *sgs1::AUR*	This study
RKH113	*MAT***a**/*MAT*α NKY1543 but *sgs1::AUR*	This study
NKY2846	*MAT***a**/*MAT*α NKY1303/NKY1543	This study
RKH225	*MAT***a**/*MAT*α RKH112/RKH113	This study
S754	*MAT***a**/*MAT*α *ura3 lys2 ho::LYS2 leu2Δ* (*asp718-ecoRI*) *arg4Δ*(*eco47III-hpaI*) *his4::URA3*-(*arg4-bgl*) *rad50S-KI81::URA3*	Bishop et al. (4)
S756	*MAT***a**/*MAT*α *ura3 lys2 ho::LYS2 leu2Δ*(*asp718-ecoRI*) *arg4Δ*(*eco47III-hpaI*) *his4::URA3*-(*arg4-nsp*) *rad50S-KI81::URA3*	Bishop et al. (4)
RKH110	*MAT***a**/*MAT*α S754 but *sgs1::AUR*	This study
RKH111	*MAT***a**/*MAT*α S756 but *sgs1*::*AUR*	This study
S1510	*MAT***a**/*MAT*α S754/S756	This study
RKH221	*MAT***a**/*MAT*α RKH110/RKH11	This study

homologues are classified into two groups, according to size. One group includes prokaryotic RecQ homologues, *E. coli* RecQ, human RecQL1, and RecQL5, which consist of about 600 to 650 amino acids; the other group includes Sgs1, Hus2/Rqh1/Rad12 of *S. pombe*, RecQL4, BLM, and WRN, consisting of about 1,400 amino acids. The latter RecQ homologues have a highly charged N- or C-terminal domain (19). A search of the entire genome of *S. cerevisiae* revealed that *SGS1* is the sole homologue of *recQ* in *S. cerevisiae*. Thus, it seems likely that *SGS1* is a functional homologue of one or several human *RECQ genes.*

To clarify the functions of Sgs1 and to obtain insight into the functions of BLM and WRN, we analyzed in detail the cause of the poor-sporulation phenotype of *sgs1* disruptants in relation to meiotic processes, including meiotic recombination.

MATERIALS AND METHODS

Yeast strains and plasmids. The origins and relevant genotypes of the strains used are listed in Table 1. The strains designated MR966 and MR93-28C (33), NKY1303 and NKY1543 (46), and S754 and S756 (*rad50S*) (4) are SK1 derivatives. Yeast manipulations were carried out as described by Sherman et al. (42). Plasmids were constructed by standard procedures (37). The full-length *SGS1* gene was isolated by PCR using a genomic DNA isolated from strain W303. PCR products were cloned into pBluescript SK(+). pYCp1305 contains the full-length *SGS1* gene (nucleotides [nt] −207 to 5558, from the *Xho*I site to the *Sac*I site) of the YCp vector, pRS314, which includes a centromere element, an autonomously replicating sequence, and a *TRP1* marker (43).

Gene disruption. The *SGS1* gene was disrupted by the one-step gene substitution method (36). A 0.7-kbp (nt 2258 to 3048) fragment containing *URA3*, *LEU2*, or *AUR* (for aureobasidin 1-C) at the *Stu*I site in the middle of the helicase domain was introduced into desired strains by a conventional transformation method. The resultant transformants were selected on synthetic complete medium (SC) plates lacking uracil or leucine or on yeast extract-peptone-adenine-dextrose (YPAD) plates containing aureobasidin A. *sgs1* null deletion disruptants (*sgs1Δ::AUR*) were made by replacing 4,365 bp of the *SGS1* sequence (nt −207 to 4158, from the *Xho*I site to the *Ehe*I site) with the aureobasidin 1-C (*AUR*) gene. The resultant transformants were selected on YPAD plates including aureobasidin A. Plasmids pNKY58 (from N. Kleckner), HT16 (from H. Ogawa), pHSS6 (from R. E. Malone), and pWL8 (from T. Weinert) were used to generate *spo13*, *mre11*, *red1*, and *rad17* disruptants, respectively. Gene disruption was confirmed by PCR or Southern blot analysis. Diploid *sgs1* disruptants of W303-1A and YPH499 were constructed using the HO plasmid on WQ701 and YQ401, respectively. In the case of SK1 background strains, MR966, MR93-28C, NKY1303, NKY1543, S754, and S756, **a** and α haploid gene disruptants were constructed and then mated to make diploids.

Sporulation and return-to-growth assay. For sporulation on plates, cells were incubated on yeast extract-peptone-dextrose (YPD) plates for 2 days and allowed to sporulate for 5 days on plates containing 1% potassium acetate (KAC plates). Sporulation was monitored with a phase-contrast microscope, and the percentage of cells forming asci was calculated. For sporulation in liquid medium, sporulation and the return-to-growth assay were performed essentially as described by Dykstra et al. (7). Cells were grown with vigorous aeration at 28°C in presporulation medium (SPS) containing 0.5% yeast extract, 1% peptone, 0.17% yeast nitrogen base without amino acids, 0.05 M potassium phthalate, 1% KAC, 0.5% ammonium sulfate, and the required amino acids. Cells were grown to a density of 2×10^7 to 5×10^7 cells/ml, washed twice with warmed 1% KAC, and suspended in warmed sporulation medium (SPM) (1% KAC, one-fifth the standard concentration of required amino acids, 0.005% Nonidet P-40) at a density of 2×10^7 cells/ml. Cells were incubated with vigorous shaking; the volume of the

TABLE 2. Poor sporulation of *sgs1* disruptants

Strain background	Genotype	Sporulation efficiency (% of asci formed)[a]
W303-1A	*SGS1*/*SGS1*	16.2 ± 3.7
	sgs1::*URA3*/*sgs1*::*URA3*	2.2 ± 1.4
YPH499	*SGS1*/*SGS1*	11.0 ± 2.1
	sgs1::*URA3*/*sgs1*::*URA3*	0.5 ± 0.3
MR966/MR93-28C	*SGS1*/*SGS1*	52.8 ± 9.1
	sgs1::*URA3*/*sgs1*::*LEU2*	4.2 ± 2.2

[a] Diploid cells incubated on YPD plates for 2 days were transferred to 1% KAC plates and incubated for 5 days to allow sporulation. Sporulation was monitored with a phase-contrast microscope.

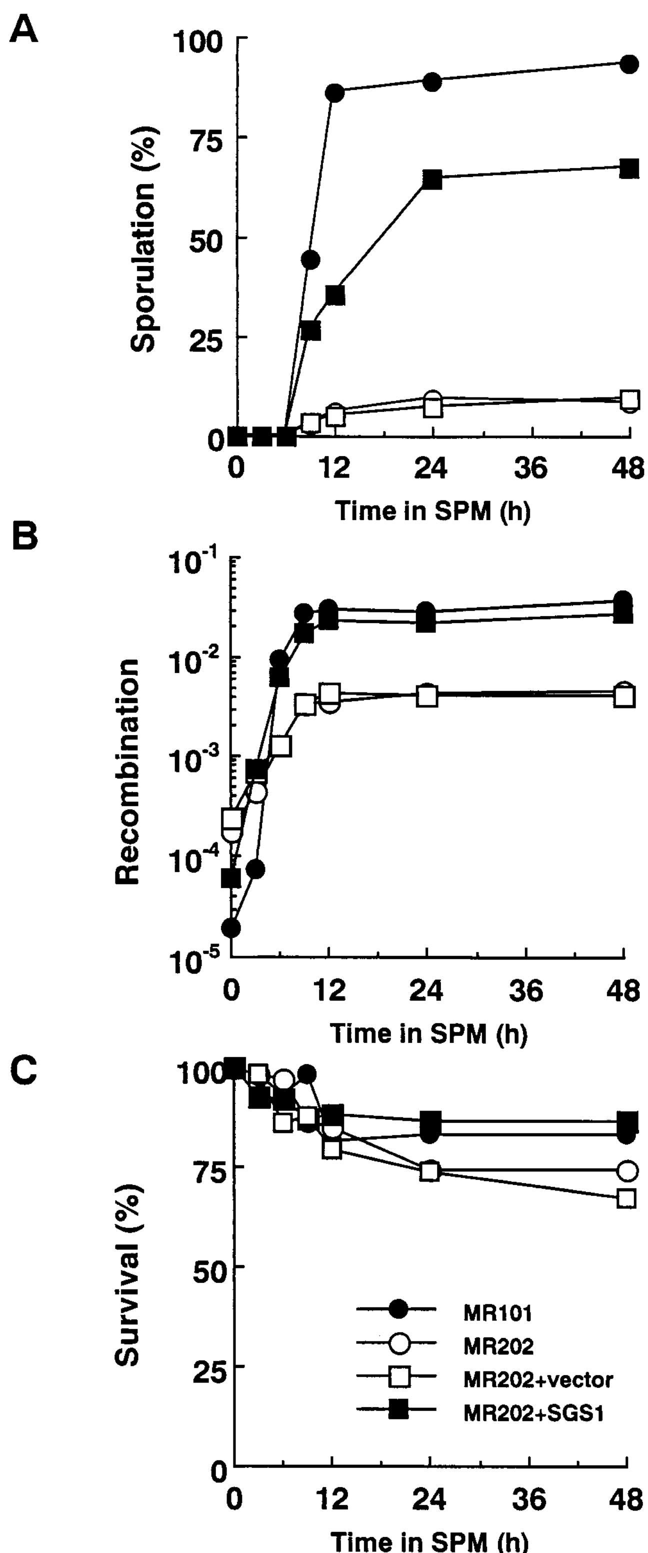

FIG. 1. Poor sporulation and reduction in the level of intergenic recombination of *sgs1* disruptants. Cells treated as described in Materials and Methods were removed after transfer to SPM. Aliquots of 1 ml were removed at various times after the shift, and sporulation, recombination, and cell viability were assessed. YCp vectors, pRS314 (vector only), and pYCp1305 (containing full-length *SGS1*) were transfected into disruptants to confirm the effect of *SGS1*. Symbols: ●, MR101 (*SGS1/SGS1*); ○, MR202 (*sgs1::URA3/sgs1::LEU2*); □, MR202 plus pRS314; ■, MR202 plus pYCp1305. (A) Sporulation was monitored with a phase-contrast microscope, and the percentage of cells which formed asci containing any spores was measured. (B) The frequency of recombination between the *his1-1* and *his1-7* alleles was measured by a return-to-growth assay. The frequency was determined by comparing the number of colonies formed on SC-His(−) plates with the number formed on SC plates after incubation for 3 days at 28°C. (C) The viability of the cells was measured by enumerating colonies that appeared on SC plates after incubation for 3 days at 28°C, taking the viability of the cells at time zero as 100%.

cell suspension was less than one-eighth the capacity of the flask to ensure good aeration. The frequency of recombination between the *his1-1* and *his1-7* alleles was examined at different times after the shifting to SPM by inoculating cells on SC plates lacking histidine [SC-His(−)] or containing histidine [SC-His(+)]. The recombination frequency was determined by comparing the number of colonies on SC-His(−) plates with the number of colonies on SC-His(+) plates.

Flow cytometry. Cells were fixed with 70% ethanol, washed with 0.2 M Tris-HCl (pH 7.5), and exposed to 0.1 mg of RNase A per ml for 3 h at 37°C. Cells were washed with 0.2 M Tris-HCl (pH 7.5), stained with 0.1 mg of propidium iodide per ml for 15 min on ice, and analyzed with a FACScan/CellFIT system (Becton Dickinson).

Preparation of DNA and detection of DSBs or physical recombinants. DNA was isolated from yeast cells using the Zymolyase method (14). For the detection of double-strand breaks (DSBs) in MR and *rad50S* strains, 5 μg of DNA samples was digested with *Xho*I and fractionated in an 0.8% agarose gel. Hybridization was performed using rapid-hyb buffer (Amersham) essentially as described by Sambrook et al. (37) and using the *Xho*I-*Nhe*I fragment from a plasmid having the YCR47C-YCR48W region in chromosome III (51a). The detection of physical recombinants of strains NKY2846 and RKH225 was performed essentially as described by Storlazzi et al. (46). For the detection of physical recombinants of RKH strains, 5 μg of DNA samples was digested with *Xho*I or *Xho*I-*Mlu*I and fractionated in a 0.6% agarose gel. Hybridization was performed using probe B (see Fig. 5A). Probes were labeled with ^{32}P using a Rediprime random primer labeling kit (Amersham). Bands were visualized and quantified with a BAS-Mac system (Fuji Film).

Site-directed mutagenesis. pYCp1306 was constructed by converting the *Pst*I site (CTGCAG) of *SGS1* in pRS314 to a *Sac*II site (CCGCGG) without a change in amino acid sequence. pYCp1309 (sgs1-hd) was constructed by replacing the *Hin*dIII-*Pst*I fragment of *SGS1* in pYCp1306 with a fragment encoding alanine (GCA) instead of lysine (AAA) at amino acid position 706 in helicase motif I. The mutagenized fragment was made by PCR, and the mutation was confirmed by DNA sequencing.

RESULTS

Poor sporulation of *sgs1* disruptants of various strains. Diploid *sgs1* disruptants were constructed using three yeast strains, W303, YPH, and MR, to investigate the function of *SGS1* in meiosis. Sporulation on KAC plates was assessed by counting the cells containing asci. The sporulation frequencies of the W303 and YPH strains on KAC plates were 16.2 and 11.0%, respectively. Those of the MR strain, a derivative of SK1, were 52.8% on KAC plates and about 90% in SPM. The sporulation frequencies of the *sgs1* disruptants derived from the W303, YPH, and MR strains were decreased by about 1/7, 1/22, and 1/13, respectively, compared with that of the corresponding wild-type cells (Table 2).

Spore formation and premeiotic DNA replication in MR strains. At 24 h after the change to SPM, the sporulation frequency of *sgs1* disruptants (MR202) reached only 9.3%, while that of wild-type cells (MR101) attained 89% (Fig. 1A). We also examined the effect of disruption of the *SGS1* gene on the frequency of recombination between heteroalleles at the *HIS1* locus during meiosis. The frequency of recombination, measured by a return-to-growth assay, was reduced by 8.2-fold in *sgs1* disruptants compared to that of wild-type cells (Fig. 1B). The increased recombination frequency of *sgs1* disruptants compared with that of wild-type cells at time zero seems to correspond to the hyperrecombination phenotype of *sgs1* disruptants during mitotic growth, as reported previously (51). The viability of *sgs1* disruptants at 24 h after the transfer to SPM was 74%, while that of wild-type cells was 84% (Fig. 1C). These poor-sporulation and reduced-recombination phenotypes were complemented with *SGS1* on either a single-copy

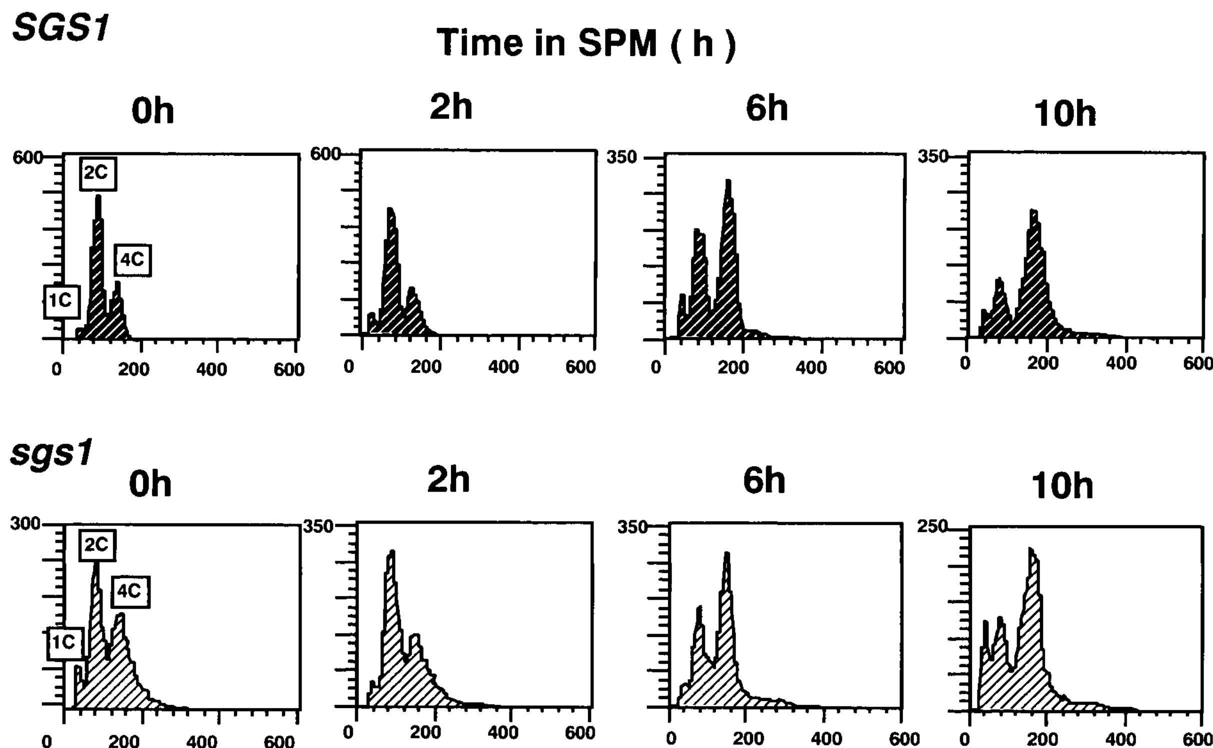

FIG. 2. Flow cytometric analysis of DNA content. MR101 (*SGS1/SGS1*) and MR202 (*sgs1::URA3/sgs1::LEU2*) cells were removed at various times after the shift to SPM and fixed with 70% ethanol. The DNA content of the samples was analyzed with a FACScan/CellFIT system.

plasmid (pYCp1305) (Fig. 1A and B) or a multicopy plasmid (data not shown).

Most of the *sgs1* disruptants were arrested at the mononucleated stage after the shift to SPM, raising the possibility that premeiotic DNA replication is affected in *sgs1* disruptants. Thus, DNA replication in *sgs1* disruptants (MR202) and wild-type cells (MR101) after transfer to SPM was monitored by flow cytometric analysis. The majority of disruptants and wild-type cells had 2C DNA before induction of sporulation (Fig. 2). At 10 h after the shift, the majority of disruptants and wild-type cells had 4C DNA, indicating that the bulk of DNA replication was completed in most cells, even disruptants. These results suggest that Sgs1 is not involved until after most of the DNA is synthesized.

Analysis of meiosis-specific DSBs in *sgs1* disruptants. The formation of DSBs is considered the initial event in meiotic recombination (2, 5, 47). We examined DSB formation during meiosis in *sgs1* disruptants. As shown in Fig. 3, transient DSB signals in the YCR47C-YCR48W region, which is the *THR4* centromere-proximal region on chromosome III, were visualized by using the YCR48W probe (51a). DSB signals were observed in wild-type cells 2 h after the shift to SPM, reached a maximum level at 4 h, and gradually decreased. In *sgs1* disruptants, the number of DSBs was considerably decreased.

To elucidate whether the formation of DSBs was really reduced in *sgs1* disruptants, we examined the accumulation of DSBs at the YCR47C-YCR48W loci in chromosome III in the *rad50S* (*rad50S-KI81*) background (4), in which the processing but not the formation of DSBs is blocked (2, 5). As shown in Fig. 4, DSBs appeared about 5 h after the shift to SPM and accumulated gradually in *rad50S* cells (S1510). In *rad50S sgs1* disruptants (RKH221), the appearance of DSBs was delayed about 1 h and the maximum level attained was slightly lower than that in *rad50S* cells.

Physical analysis of the recombination products in *sgs1* disruptants during meiosis. The physical recombinants at the *his4-LEU2* loci were examined (Fig. 5A) (46). Bands corresponding to crossover-type recombination can be discriminated from parental fragments by restriction site polymorphism of the *Xho*I site between homologous chromosomes. The bands derived from crossover-type recombination were detected 8 h after the shift to SPM in wild-type cells and *sgs1* disruptants. The intensities of the bands reached 20 and 13% that of the parental bands by 12 h for wild-type cells and *sgs1* disruptants, respectively (Fig. 5B). Bands derived from noncrossover-type recombination (gene conversion) are detectable with the same specific probe by Southern blotting with DNA samples digested with *Xho*I and *Mlu*I (46). We examined noncrossover-type recombination and found that it occurred in *sgs1* disruptants as well as in wild-type cells (data not shown).

Analysis of *SGS1* function during sporulation by deletion of *SPO13* and *MRE11*. Spo13 is required for meiosis I, and deletion of the *SPO13* gene results in a single meiosis II-like division, producing dyad asci, and rescues the meiotic lethality of early recombination-deficient mutants by bypassing meiosis I division (29). Mre11 is required in the early stages of meiotic recombination, including DSB formation, and *mre11* mutants produce nonviable spores (1, 15). To analyze the function of *SGS1* during sporulation, we constructed double disruptants, *spo13 sgs1*, *mre11 sgs1*, and *spo13 mre11* (Table 1), and examined whether these cells were capable of sporulation. These double disruptants were analyzed for mitotic recombination frequency, sporulation, spore viability, and meiotic recombination frequency, as measured by the return-to-growth assay (Table 3). *spo13 mre11* double disruptants produced dyad asci with 63.2% spore viability and showed a remarkably reduced meiotic recombination frequency, as reported by Ajimura et al. (1). For *sgs1 spo13* double disruptants, 10% of the cells formed

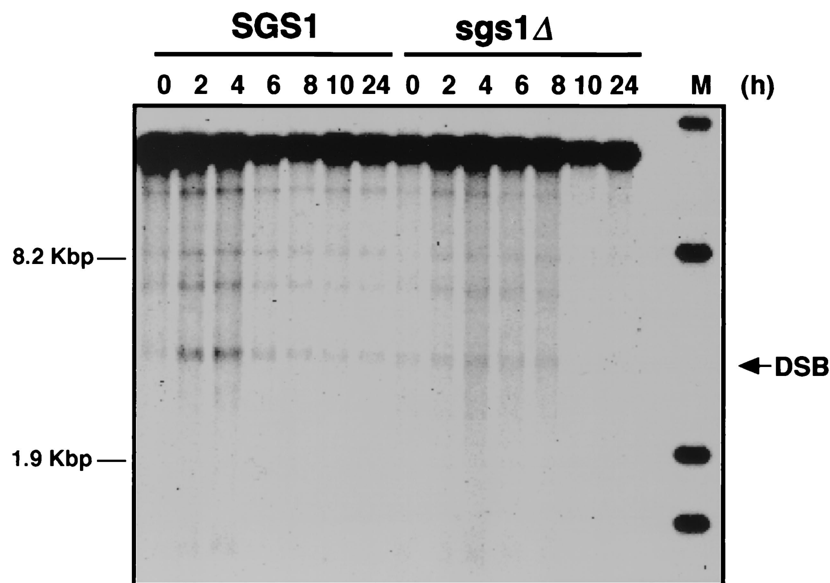

FIG. 3. Detection of DSB in *sgs1* disruptants. MR101 (*SGS1*) and MR301 (*sgs1Δ::AUR*) cells were treated as described in Materials and Methods and harvested at various times after transfer to SPM. DNA samples were isolated from the cells, and aliquots (5 μg) of the DNA were separated on an agarose gel. DSB signals in the YCR47C-YCR48W region were analyzed by Southern blotting.

dyad asci at 24 h after the shift to SPM; this value was the same as that for *sgs1* disruptants and was 5.2- and 3.3-fold lower than those for *spo13* and *spo13 mre11* cells, respectively. The spore viability of *sgs1 spo13* cells was 5.0- and 3.4-fold lower than those of *sgs1* and *spo13* cells, respectively. Very few *sgs1 mre11* double disruptants sporulated, and the spores were nonviable. All the single and double disruptants except for *spo13* showed mitotic hyperrecombination.

Either the *red1* or the *rad17* mutation partially alleviates the prophase arrest of *sgs1* disruptants. The facts that the majority of *sgs1* disruptants remained in the mononucleated stage after transfer to SPM and showed relatively high viability indicate that the defect due to the dysfunction of Sgs1 produces a signal to arrest cells at or before meiosis I. Thus, we examined the possibility that mononucleated *sgs1* cells were arrested by meiotic checkpoint control.

The disruption of either the *RED1* or the *RAD17* gene, both of which are involved in meiotic checkpoint control (25, 52), resulted in partial alleviation of the poor-sporulation phenotype of *sgs1* disruptants (Fig. 6).

Missense mutation in the ATP binding motif of *SGS1* affects sensitivity to MMS and HU but not sporulation. DNA helicase motif I is known to be involved in ATP binding. Lu et al. reported that a missense mutation of Sgs1 in helicase motif I at amino acid position 706, from lysine to alanine, abolished helicase activity (23). To determine the requirement of helicase activity for various functions of Sgs1, a plasmid carrying an *sgs1* gene encoding Sgs1 having the same missense mutation (sgs1-hd) was transformed into *sgs1* disruptants. As shown in Fig. 7, sgs1-hd was not able to complement the sensitivity to MMS or HU of the *sgs1* partial disruptant (MR202) or null mutant (MR301). In contrast, the poor-sporulation phenotype and reduced frequency of meiotic recombination monitored by the return-to-growth assay were rescued by sgs1-hd in the *sgs1* null mutant (MR301) as well as the partial disruptant (MR202) (Table 4). The increased recombination in *sgs1* disruptants at time zero, which corresponds to mitotic recombination, was not suppressed by sgs1-hd.

DISCUSSION

It has been reported that *sgs1* mutants show a poor-growth phenotype in the *top1* mutant background (23), suppression of *top3*-associated poor growth (11), hypersensitivity to HU (53) and MMS (10), defects in faithful chromosome segregation in mitosis as well as meiosis (50), mitotic hyperrecombination phenotypes (31, 51, 53), poor sporulation (12, 51), and premature aging (44). In this study, we analyzed in detail the poor-sporulation phenotype of *sgs1* disruptants in relation to meiotic recombination.

Meiotic recombination in an *sgs1* mutant examined by physical analysis. We observed poor-sporulation phenotypes in *sgs1* disruptants from several strains with different genetic backgrounds (Table 2), as reported previously (12, 50, 51). The apparent frequency of meiotic recombination, measured by a return-to-growth assay, was decreased severalfold in *sgs1* disruptants compared with wild-type cells (Fig. 1). Thus, we examined recombination intermediates of *sgs1* disruptants by physical analysis. Although the number of DSBs was reduced severalfold in *sgs1* disruptants compared with wild-type cells, almost the same number of DSBs accumulated in *sgs1 rad50S* cells as in *rad50S* cells. Similar results were reported for *red1* and *mek1*/*mre4* mutants (52). In *red1* and *mek1*/*mre4* mutants, the steady-state level of DSBs was reduced, but the number of DSBs that accumulated in *red1 rad50S* and *mek1 rad50S* cells was almost the same as that in *rad50S* cells. Xu et al. proposed that in *red1* and *mek1*/*mre4* mutants, the kinetics of DSB formation are negatively regulated by meiosis-specific surveillance mechanisms, and the conversion of DSBs to double Holliday junctions would be one of the most important checkpoints (52). Thus, we speculated that the kinetics of DSB formation, rather than the machinery to form DSBs, are somehow affected in *sgs1* disruptants.

Meiotic recombination products of either the crossover type or the gene conversion type appeared in *sgs1* disruptants as well as wild-type cells. Since the appearance of recombinant molecules does not necessarily require the resolution of Hol-

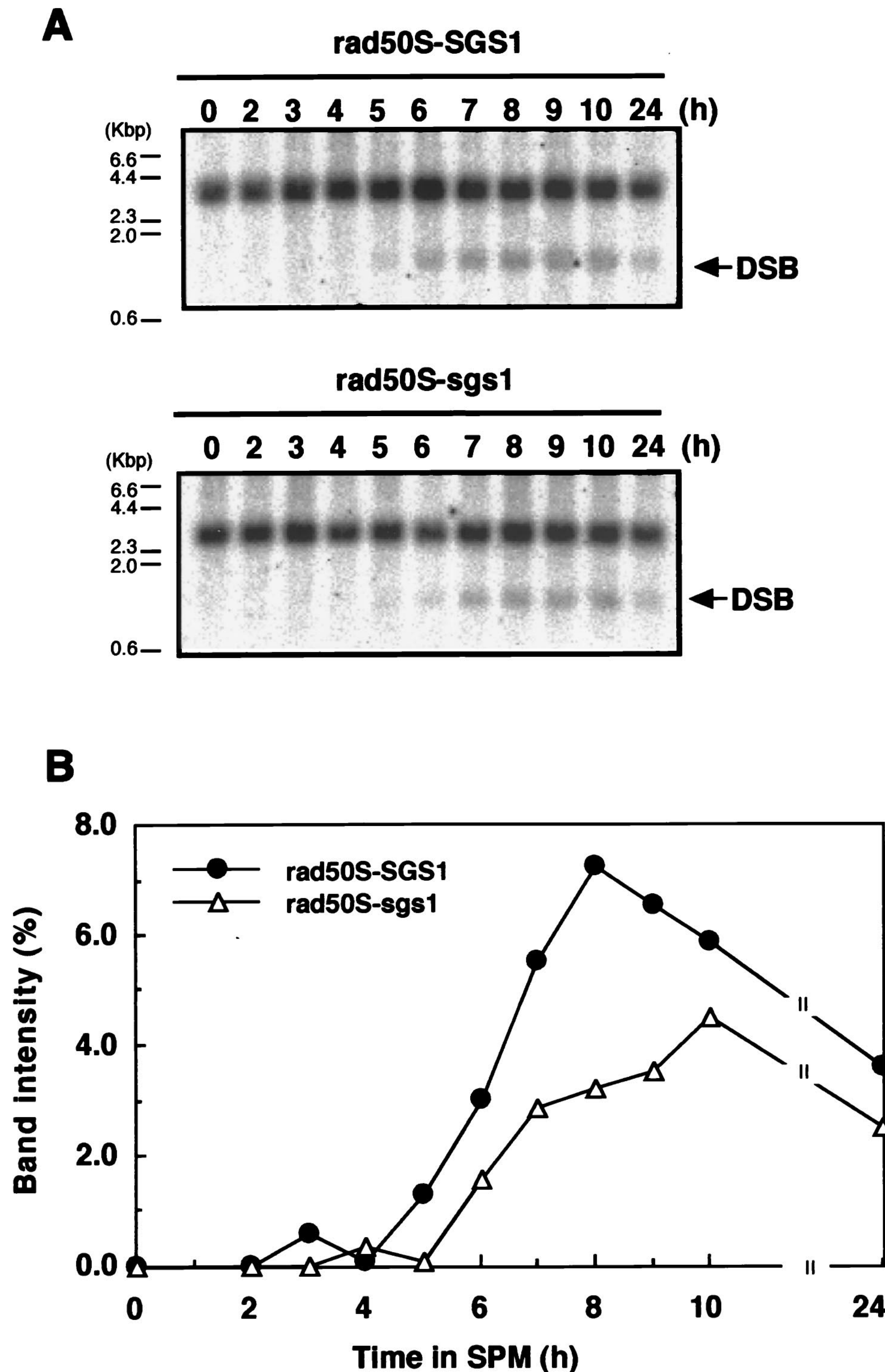

FIG. 4. Accumulation of DSBs in *rad50S sgs1* mutants. (A) Wild-type cells and *sgs1* disruptants with the *rad50S* background, S1510 (*SGS1*) and RKH221 (*sgs1::AUR*), were treated as described in Materials and Methods and harvested at various times after transfer to SPM. DNA was isolated from the cells, and aliquots (5 μg) of the DNA were separated on an agarose gel. DSB signals in the YCR47C-YCR48W region were analyzed by Southern blotting. (B) The intensity of the band corresponding to the DSB signal was quantified and expressed as a percentage of the intensity of all bands in the lane.

liday junctions, the possibility remains that the defect of Sgs1 function affects the recombination process itself.

The frequency of meiotic recombination measured by the return-to-growth assay was decreased severalflold in *sgs1* disruptants compared with wild-type cells (Fig. 1). Watt et al. reported no decrease in the meiotic recombination frequency in spores formed by *sgs1* mutants (51). This discrepancy can be explained by the differences in the populations analyzed; that is, Watt et al. dealt with only viable spores, and the return-to-growth assay dealt with the whole population, most of which

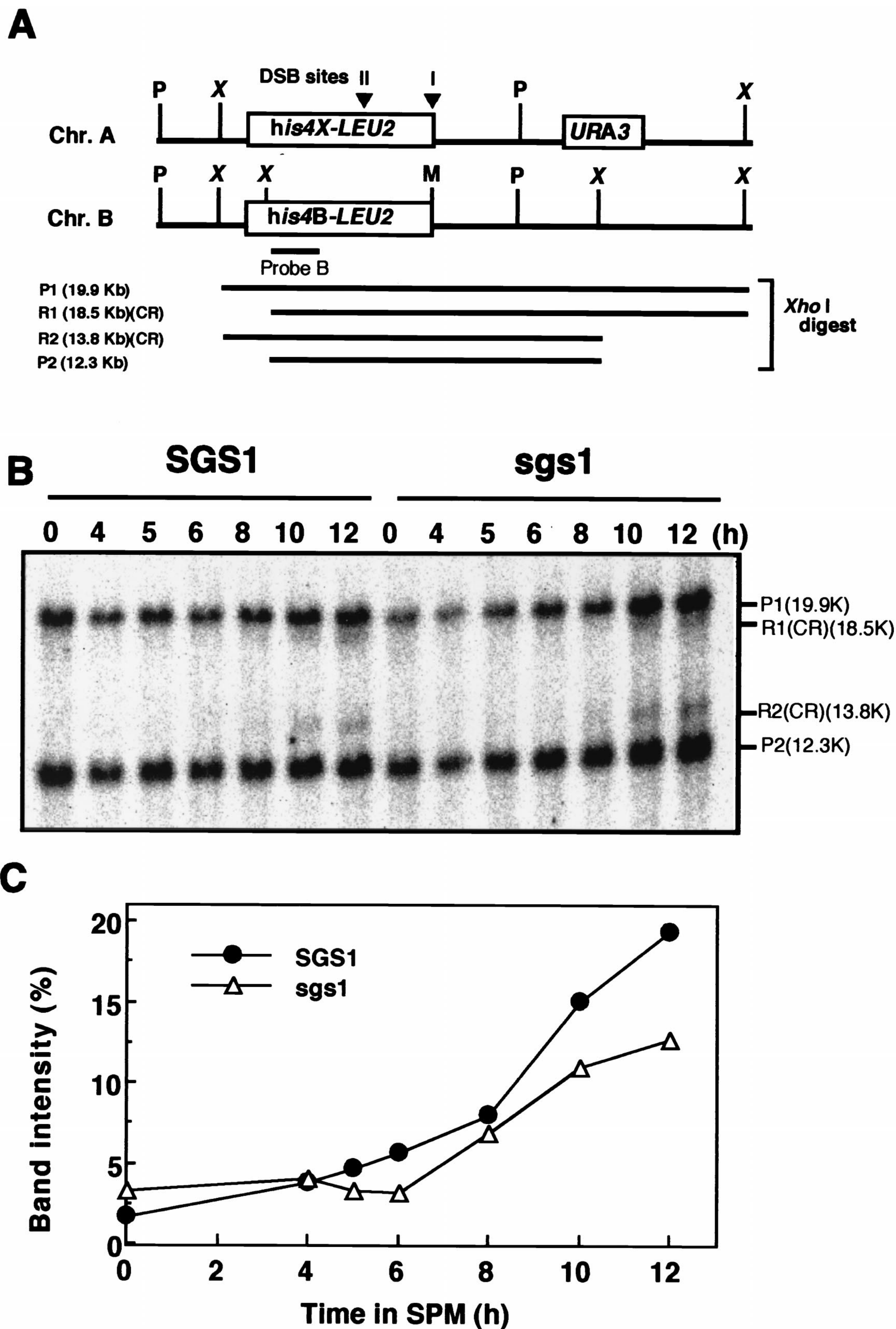

FIG. 5. Detection of crossover-type recombination products in *sgs1* disruptants. (A) Map of the *his4 LEU2* locus on chromosome III. P, X, and M, restriction sites for *Pst*I, *Xho*I, and *Mlu*I, respectively; P1 and P2, fragments derived from the parental DNAs after digestion with *Xho*I; R1 and R2, fragments derived from crossover-type (CR) recombination products. (B) NKY2846 (*SGS1*) and RKH225 (*sgs1::AUR*) cells treated as described in Materials and Methods were harvested at various times after transfer to SPM. DNA was isolated from the cells, and aliquots (5 μg) of *Xho*I-digested DNA were analyzed by Southern blotting. The probe used was derived from pNKY155. (C) The intensity of the bands corresponding to R1 and R2 was quantified and expressed as a percentage of the intensity of all bands in the lane.

TABLE 3. Sporulation, spore viability, and recombination in *sgs1 spo13* and *sgs1 mre11* double disruptants

Strain	Genotype	Mitotic recombination (His$^+$ frequency 10^{-5})[a]	Sporulation (%) at[b]:		Spore viability[c]	Meiotic recombination (His$^+$ frequency, 10^{-3}) at 24 h[d]
			24 h	96 h		
MR101	*SGS1 SPO13 MRE11*	5.8	81	88	75/84 (89.3)	37.9
ds1	*sgs1*Δ	57.6	8	10	72/124 (58.1)	3.9
ds13	*spo13*	9.5	**52**	**79**	48/120 (40.0)	6.5
ds1s13	*sgs1*Δ *spo13*	56.3	**10**	**15**	15/128 (11.7)	3.9
ds1m11	*sgs1*Δ *mre11*	38.8	0.6	0.1	0/120 (0.0)	0.2
ds13m11	*spo13 mre11*	44.6	**33**	**55**	86/136 (63.2)	0.1

[a] The frequency of mitotic recombination between the *his1-1* and *his1-7* alleles was determined by comparing the number of colonies on SC-His(−) plates with the number on SC plates.

[b] Diploid cells were precultured in SPS and inoculated into SPM at a density of 2×10^7 cells/ml with vigorous shaking. Aliquots of 1 ml were taken at the times indicated, sporulation was monitored at 24 or 96 h after the shift to SPM with a phase-contrast microscope, and the percentage of tetrad asci or dyad asci (boldface values) for *spo13* disruptants relative to total cell number was calculated.

[c] Spore viability was determined after dissection by dividing the number of spores forming colonies by the total number dissected. Numbers in parentheses are percentages.

[d] The frequency of meiotic recombination between the *his1-1* and *his1-7* alleles was determined at 24 h after the shift to SPM by comparing the number of colonies on SC-His(−) plates with the number on SC plates.

was not able to form spores (Fig. 1). However, the physical assay of amounts of meiotic recombinants showed little difference between *sgs1* and wild-type cells. The decrease in meiotic recombination in the return-to-growth assay was also observed in *top3* disruptants, and the low level of meiotic recombination in this assay was explained to be due to the loss of viability of meiotic cells following DSB formation in *top3* mutants (12). However, this explanation cannot be applied for the *sgs1* disruptants in this study, because only a slight reduction in viability was observed with *sgs1* disruptants in the return-to-growth assay (Fig. 1).

In most *sgs1* disruptants, meiosis I is not bypassed upon disruption of *SPO13*. On deletion of the *SPO13* gene, meiosis I is bypassed and dyad asci are produced (29). Deletion of the *SPO13* gene in a *rad52*, *rad50S*, or *dmc1* background, which is defective at a point after DSB formation, produced dead or very-low-viability dyad asci (4, 27). The viability of spores formed in either *rad52*, *rad50S*, or *dmc1* single mutants also was very low (4, 27). Disruptants of the genes required before or for DSB formation during meiotic recombination, such as *spo11*, *mre11*, and *rad50*, sporulate with almost normal efficiency but produce nonviable spores, and disruption of *SPO13* in *spo11*, *mre11*, or *rad50* mutants results in the formation of viable dyad asci without meiotic recombination (1, 21, 26).

In fact, *spo13 mre11* mutants sporulated and produced dyad asci containing viable spores (Table 3), as reported previously (1). The results obtained with *sgs1* and *sgs1 spo13* disruptants were quite different from those obtained with the above mutants. The *sgs1* disruptants showed low sporulation efficiency, but the spores produced showed relatively high viability, and the disruption of *SPO13* in *sgs1* disruptants did not alleviate the low sporulation efficiency of the *sgs1* disruptants. Thus, it must be emphasized that the majority of *sgs1* disruptants have

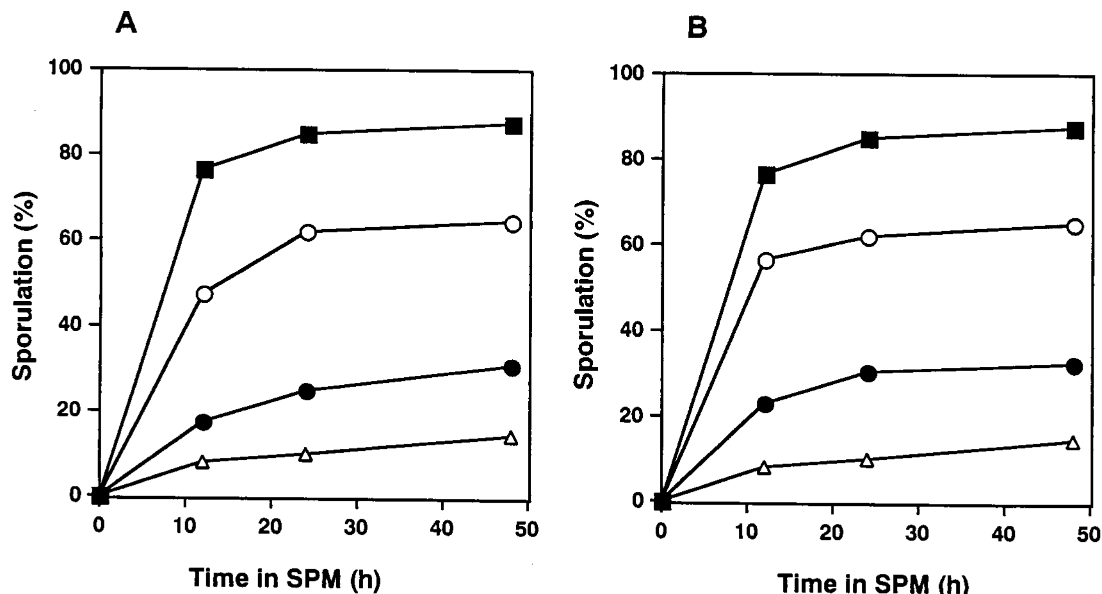

FIG. 6. Partial suppression of poor sporulation of *sgs1* null mutants by the deletion of *RED1* or *RAD17* gene function. Cells treated as described in Materials and Methods were harvested after transfer to SPM. Aliquots (1 ml) were removed at various times after the shift to SPM, and the cells were examined for sporulation. (A) Symbols: ■, wild-type cells (MR101); △, *sgs1* cells (MR301); ○, *red1* cells (dr1); ●, *red1 sgs1* cells (dslr1). (B) Symbols: ■, wild-type cells (MR101); △, *sgs1* cells (MR301); ○, *rad17* cells (dr17); ●, *rad17 sgs1* cells (dslr17).

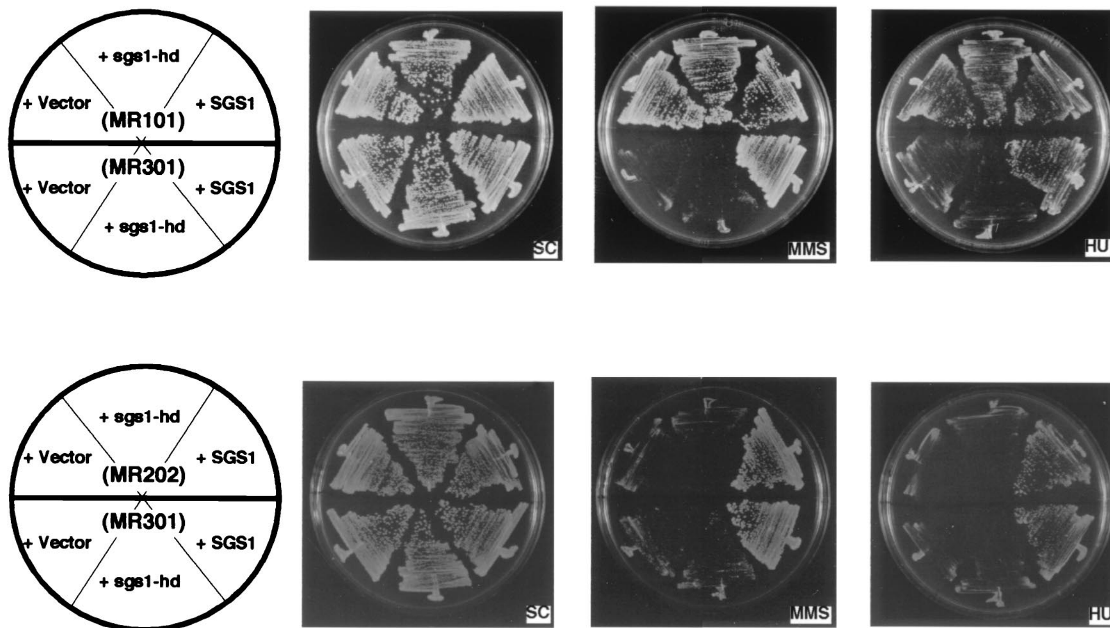

FIG. 7. An *sgs1* gene coding for a protein defective in DNA helicase activity cannot complement the MMS and HU sensitivities of *sgs1* disruptants. Wild-type cells (MR101), *sgs1* disruptants (MR202), and *sgs1* null deletion disruptants (MR301) were transformed with pRS314 (vector only), pYCp1309 (sgs1-hd), and pYCp1305 (containing full-length *SGS1*). Cells were streaked onto SC plates containing MMS (0.02%) or HU (100 mM) and were photographed after 3 days at 28°C.

a defect that renders them unable to bypass meiosis I on disruption of *SPO13*. A similar result was obtained with *top3* disruptants (12). A small portion of the *sgs1* population underwent meiosis almost normally, and the spores showed relatively high viability. However, a defect in segregation was observed in this population, since a considerable number of the asci formed by *sgs1* disruptants contained odd numbers of spores (data not shown), as reported previously (51).

The *rad50S* mutants, unable to process DSBs, showed a poor-sporulation phenotype, and both *dmc1* and *top2* mutants, which are defective in reciprocal recombination and the resolution of recombination intermediates, respectively, were arrested at meiotic prophase under meiosis-specific checkpoint control. The introduction of mutations in either *MRE11* or *RAD50* in the above mutants to form *mre11 rad50S*, *rad50 dmc1*, and *rad50 top2*, which eliminates DSB formation, resulted in the restoration of sporulation and the production of dead spores (1, 4, 35). Thus, if Sgs1 is required only after DSB formation in meiotic recombination, *sgs1 mre11* double mutants should show phenotypes similar to those of *mre11* single mutants. However, this was not the case, because the reduced level of spore formation by *sgs1* mutants could not be alleviated by introducing the *MRE11* mutation, that is, eliminating DSBs. Similar results were obtained for *top3* mutants by Gangloff et al. (12), who reported that disruption of *SPO11*, which encodes the enzyme essential to form DSBs (17), could not alleviate the *top3* sporulation defect.

Arrest of *sgs1* disruptants in the mononucleated stage is caused partially by meiotic checkpoint function. The facts that the majority of *sgs1* disruptants remained in the mononucle-

TABLE 4. Analysis of Sgs1 functions[a]

Strain	Plasmid	Mitotic recombination (His$^+$ frequency, 10^{-5}) at 0 h	Sporulation (% of asci formed) at: 12 h	Sporulation (% of asci formed) at: 24 h	Meiotic recombination (His$^+$ frequency, 10^{-3}) at 24 h
MR101 (WT)	Vector	2.7	82	89	27.8
	SGS1	3.1	82	89	26.1
	sgs1-hd	3.5	81	89	24.1
MR202 (*sgs1*)	Vector	23.4	4.9	7.4	4.2
	SGS1	6.0	36	64	22.3
	sgs1-hd	16.3	23	50	15.5
MR301 (*sgs1*Δ)	Vector	20.1	7.0	9.2	5.5
	SGS1	2.2	25	58	24.8
	sgs1-hd	27.2	28	51	17.3

[a] Wild-type (WT) cells (MR101), *sgs1* disruptants (MR202), and *sgs1* null deletion disruptants (MR301) were transformed with pRS314 (vector only), pYCp1305 (containing full-length *SGS1*), and pYCp1309 (sgs1-hd). Sporulation was monitored with a phase-contrast microscope at 12 and 24 h after the shift to SPM. The frequency of recombination between the *his1-1* and *his1-7* alleles was examined at 0 h (for mitotic recombination) and 24 h (for meiotic recombination by a return-to-growth assay) after the shift to SPM.

ated stage after transfer to SPM and showed relatively high viability indicate that the defect due to the dysfunction of Sgs1 produces a signal to arrest cells at or before meiosis I. Gangloff et al. (12) showed that *sgs1* disruptants of strain W303 could sporulate but that the process was delayed and inefficient compared to that of wild-type cells, and they argued that the Sgs1 defect generates a checkpoint signal. We also monitored the sporulation of *sgs1* disruptants (MR301) for up to 12 days. The percentage of asci that were formed gradually increased but at 12 days was still only 18.5%, compared with 85% for wild-type cells. For *dmc1* mutants, lacking Dmc1, which is the meiosis-specific Rad51 homologue, cells with recombination intermediates arrested in the prophase without a loss of viability (4). The disruption of *RED1* in *dmc1* mutants released the prophase arrest of the *dmc1* mutants and restored spore formation (52). Cells lacking Red1, which is the meiosis-specific component of the axial element, failed to form a synaptonemal complex (34) and to check aberrant DNA recombination (52). In addition, the disruption of *RAD17* in *dmc1* mutants alleviated the prophase arrest of the *dmc1* mutants (25), suggesting the existence of single-stranded DNA regions in uncompleted recombination intermediates in *dmc1* cells, because Rad17 is able to sense single-stranded DNA regions (24).

The disruption of either *RED1* or *RAD17* in *sgs1* disruptants partially alleviated the poor-sporulation phenotype of *sgs1* mutants. The reason why the effect was partial is not clear. One explanation is that cells in which the phenotype was not alleviated by the disruption of either *RED1* or *RAD17* have defects that cause the suppression and that can be alleviated only by disruption of both *RED1* and *RAD17* or defects that are sensed by sensors other than Red1 and Rad17. Another is that cells in which the phenotype was alleviated by the *red1* or *rad17* mutation are of different populations. Thus, it seems that *sgs1* disruptants are arrested at multiple points rather than at a single unique point. The partial suppression caused by the disruption of *RAD17* indicates the existence of single-stranded DNA regions in a portion of the arrested population of *sgs1* cells (24, 25). It seems likely that *sgs1* disruptants contain incomplete homologous recombination intermediates in which single-stranded DNA regions exist. The suppression of the late S/G_2 delay of *top3* mutants by an *SGS1* mutation suggests that both Sgs1 and Top3 are involved in the late stage of DNA replication in the mitotic cell cycle (11). Although premeiotic DNA synthesis followed a normal time course even in *sgs1* disruptants (Fig. 2), the possibility cannot be excluded that DNA replication is inhibited at a late stage and single-stranded DNA regions remain in *sgs1* disruptants.

Relationship between Top3 and Sgs1 functions in meiosis. The protein encoded by *SGS1* was shown to have DNA helicase activity (3, 23). It has been reported that a missense mutation of lysine to alanine at amino acid position 706 abolishes the DNA helicase activity of Sgs1 in vitro (23). The plasmid carrying the gene with this missense mutation, sgs1-hd, rescued neither MMS sensitivity nor HU sensitivity in *sgs1* disruptants, indicating that repair of certain types of DNA damage requires the DNA helicase activity of Sgs1 (Fig. 7). Similar results were reported recently (10). In addition, sgs1-hd could suppress neither elevated homologous recombination between heteroalleles (Table 4) nor elevated unequal sister chromatid exchanges in *sgs1* mutants (31). In contrast, it was reported that the *sgs1-hd* allele behaves just like the wild-type allele: it decreases the rate of growth of *top3 sgs1* mutants and improves the poor growth of *top1 sgs1* mutants (23). In other words, DNA helicase activity is not required for complementation of *top1*- and *top3*-related *sgs1* phenotypes. In addition, we found that sgs1-hd almost completely rescued the poor-sporulation phenotype and the reduced frequency of meiotic recombination of *sgs1* disruptants. Thus, two different mechanisms underlie the functions of Sgs1; one requires DNA helicase activity, and the other does not. This finding should help us to analyze the molecular mechanisms producing the pleiotropic phenotypes of *sgs1* disruptants.

Budding yeast Top3 has a weak ability to relax negatively supercoiled double-stranded DNA and preferentially binds to single-stranded DNA regions (18). Both genetic and physical interactions have been demonstrated for Sgs1 and Top3, indicating that these proteins function as a complex in the mitotic cell cycle (11). Gangloff et al. (11) proposed that movement of the Sgs1 helicase along the DNA melts the duplex, providing a preferential single-stranded substrate for Top3, and that the Sgs1-Top3 complex might act as a reverse gyrase (6). The observation that Sgs1 devoid of DNA helicase activity could carry out its role in sporulation indicates that the postulated reverse gyrase activity of the Sgs1-Top3 complex is dispensable from the meiotic process, because helicase activity is essential for introducing positive supercoils. Thus, it seems likely that one of the possible functions of Sgs1 in the meiotic process is to recruit Top3. In this context, it is interesting that mouse *Top3*α and *Top3*β as well as *Blm* mRNAs were highly expressed in the testis and that the levels of their expression in the testis were increased simultaneously and markedly by 17 days after birth, when numbers of the cells in pachytene phase increase (39, 40, 41). In addition, it was reported recently that BLM, one of the Sgs1 homologues in higher eukaryotes, was localized on mouse meiotic chromosomes during meiotic DNA recombination (49). Taken together with the report that male BS patients are infertile (16), these results suggest that BLM plays roles in meiotic recombination.

Recently, the sporulation of *top3* mutants as well as *sgs1* mutants has been reported (12). Although the frequency of sporulation of *sgs1* mutants is low, it is higher than that of *top3* mutants, which make no spore. In addition, deletion of the *SGS1* gene restores sporulation in *top3* mutants to a level below that in *sgs1* single mutants. Thus, Sgs1 does more than simply recruit Top3; an unknown protein has the ability to recruit Top3, or Top3 itself can access the meiotic chromosome at a low efficiency.

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REFERENCES

1. **Ajimura, M., S.-H. Leem, and H. Ogawa.** 1993. Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. Genetics **133:**51–66.
2. **Alani, N., R. Padmore, and N. Kleckner.** 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:**419–436.
3. **Bennett, R. J., J. A. Sharp, and J. C. Wang.** 1998. Purification and characterization of the Sgs1 DNA helicase activity of *Saccharomyces cerevisiae*. J. Biol. Chem. **273:**9644–9650.
4. **Bishop, D. K., D. Park, L. Xu, and N. Kleckner.** 1992. *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:**439–456.
5. **Cao, L., E. Alani, and N. Kleckner.** 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:**1089–1101.
6. **Confalonieri, F., C. Elie, M. Nadal, C. B. de la Tour, P. Forterre, and M.**

Duguet. 1993. Reverse gyrase: a helicase-like domain and a type I topoisomerase in the same polypeptide. Proc. Natl. Acad. Sci. USA **90:**4753–4757.

7. **Dykstra, C. C., K. Kitada, A. B. Clark, R. K. Hamatake, and A. Sugino.** 1991. Cloning and characterization of *DST2*, the gene for DNA strand transfer protein β from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**2583–2592.
8. **Ellis, N. A., J. Groden, T.-Z. Ye, J. Straughen, D. J. Lennon, S. Ciocci, M. Proytcheva, and J. German.** 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. Cell **83:**655–666.
9. **Epstein, C. J., G. M. Martin, A. L. Schultz, and A. G. Motulsky.** 1966. Werner's syndrome: a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Medicine **45:**177–222.
10. **Frei, C., and S. M. Gasser.** 2000. The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci. Genes Dev. **14:**81–96.
11. **Gangloff, S., J. P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein.** 1994. The yeast type 1 topoisomerase Top3 interacts with Sgs1, a DNA helicase homologue: a potential eukaryotic reverse gyrase. Mol. Cell. Biol. **14:**8391–8398.
12. **Gangloff, S., B. deMassy, L. Arthur, R. Rothstein, and F. Fabre.** 1999. The essential role of yeast topoisomerase III in meiosis depends on recombination. EMBO J. **18:**1701–1711.
13. **German, J.** 1993. Bloom syndrome: a Mendelian prototype of somatic mutational disease. Medicine **72:**393–406.
14. **Goyon, C., and M. Lichten.** 1993. Timing of molecular events in meiosis in *Saccharomyces cerevisiae*: stable heteroduplex DNA is formed late in meiotic prophase. Mol. Cell. Biol. **13:**373–382.
15. **Johzuka, K., and H. Ogawa.** 1995. Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. Genetics **139:**1521–1532.
16. **Kauli, R., R. Prager-Lewin, H. Kaufman, and Z. Laron.** 1977. Gonadal function in Bloom's syndrome. Clin. Endocrinol. **6:**285–289.
17. **Keeney, S., C. N. Giroux, and N. Kleckner.** 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:**375–384.
18. **Kim, R. A., and J. C. Wang.** 1992. Identification of the yeast *TOP3* gene product as a single strand-specific DNA topoisomerase. J. Biol. Chem. **267:** 17178–17185.
19. **Kitao, S., I. Ohsugi, K. Ichikawa, M. Goto, Y. Furuichi, and A. Shimamoto.** 1998. Cloning of two new human helicase genes of the RecQ family: biological significance of multiple species in higher eukaryotes. Genomics **54:** 443–452.
20. **Kitao, S., A. Shimamoto, M. Goto, R. W. Miller, W. A. Smithson, N. M. Lindor, and Y. Furuichi.** 1999. Mutations in *RECQL4* cause a subset of cases of Rothmund-Thomson syndrome. Nat. Genet. **22:**82–84.
21. **Klapholz, S., C. S. Waddell, and R. E. Esposito.** 1985. The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:**187–216.
22. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:**401–465.
23. **Lu, J., J. R. Mullen, S. J. Brill, S. Kleff, A. M. Romeo, and R. Sternglanz.** 1996. Human homologues of yeast helicase. Nature **383:**678–679.
24. **Lydall, D., and T. Weinert.** 1995. Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. Science **270:**1488–1491.
25. **Lydall, D., Y. Nikolsky, D. K. Bishop, and T. Weinert.** 1996. A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:**840–843.
26. **Malone, R. E., and R. E. Esposito.** 1981. Recombinationless meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1:**891–901.
27. **Mao-Draayer, Y., A. M. Galbraith, D. L. Pittman, M. Cool, and R. E. Malone.** 1996. Analysis of meiotic recombination pathways in the yeast *Saccharomyces cerevisiae*. Genetics **144:**71–86.
28. **Martin, G. M.** 1977. Cellular aging-clonal senescence. Am. J. Pathol. **89:** 484–511.
29. **McCarroll, R. M., and R. E. Esposito.** 1994. *SPO13* negatively regulates the progression of mitotic and meiotic nuclear division in *Saccharomyces cerevisiae*. Genetics **138:**47–60.
30. **Nakayama, K., N. Irino, and H. Nakayama.** 1985. The *recQ* gene of *Escherichia coli* K12: molecular cloning and isolation of insertion mutants. Mol. Gen. Genet. **200:**266–271.
31. **Onoda, F., M. Seki, A. Miyajima, and T. Enomoto.** 2000. Elevation of sister chromatid exchange in *Saccharomyces cerevisiae sgs1* disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene. Mutat. Res. **459:**203–209.
32. **Puranam, K. L., and P. J. Blackshear.** 1994. Cloning and characterization of RecQL, a potential human homologue of the *Escherichia coli* DNA helicase RecQ. J. Biol. Chem. **269:**29838–29845.
33. **Resnick, M. A., A. Sugino, J. Nitiss, and T. Chow.** 1984. DNA polymerase, deoxyribonuclease, and recombination during meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**2811–2817.
34. **Rockmill, B., and G. S. Roeder.** 1990. Meiosis in asynaptic yeast. Genetics **126:**563–574.
35. **Rose, D., W. Thomas, and C. Holm.** 1990. Segregation of recombined chromosomes in meiosis I requires DNA topoisomerase II. Cell **60:**1009–1017.
36. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
38. **Seki, M., H. Miyazawa, S. Tada, J. Yanagisawa, T. Yamaoka, S. Hoshino, K. Ozawa, T. Eki, M. Nogami, K. Okumura, H. Taguchi, F. Hanaoka, and T. Enomoto.** 1994. Molecular cloning of cDNA encoding human DNA helicase Q1 which has homology to *Escherichia coli* RecQ helicase and localization of the gene at chromosome 12p12. Nucleic Acids Res. **22:**4566–4573.
39. **Seki, T., M. Seki, T. Katada, and T. Enomoto.** 1998. Isolation of a cDNA encoding mouse DNA topoisomerase III which is highly expressed at the mRNA level in the testis. Biochim. Biophys. Acta **1396:**127–131.
40. **Seki, T., M. Seki, R. Onodera, T. Katada, and T. Enomoto.** 1998. Cloning of cDNA encoding a novel mouse DNA topoisomerase III (Topo IIIβ) possessing negatively supercoiled DNA relaxing activity, whose message is highly expressed in the testis. J. Biol. Chem. **273:**28553–28556.
41. **Seki, T., W.-S. Wang, N. Okumura, M. Seki, T. Katada, and T. Enomoto.** 1998. cDNA cloning of mouse BLM gene, the homologue to human Bloom's syndrome gene, which is highly expressed in the testis at the mRNA level. Biochim. Biophys. Acta **1398:**377–381.
42. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
43. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
44. **Sinclair, D. A., K. Mills, and L. Guarente.** 1997. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. Science **277:**1313–1316.
45. **Stewart, E., C. R. Chapman, F. Al-Khodairy, A. M. Carr, and T. Enoch.** 1997. *rqh1+*, a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible S phase arrest. EMBO J. **16:**2682–2692.
46. **Storlazzi, A., L. Xu, L. Cao, and N. Kleckner.** 1995. Crossover and noncrossover recombination during meiosis: timing and pathway relationships. Proc. Natl. Acad. Sci. USA **92:**8512–8516.
47. **Sun, H., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:**87–90.
48. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56:**619–630.
49. **Walpita, D., A. W. Plug, N. F. Neff, J. German, and T. Ashley.** 1999. Bloom's syndrome protein, BLM, colocalizes with replication protein A in meiotic prophase nuclei of mammalian spermatocytes. Proc. Natl. Acad. Sci. USA **96:**5622–5627.
50. **Watt, P. M., E. J. Louis, R. H. Borts, and I. D. Hickson.** 1995. Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell **81:**253–260.
51. **Watt, P. M., I. D. Hickson, R. H. Borts, and E. J. Louis.** 1996. *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics **144:** 935–945.

51a. **Wu, T.-C., and M. Lichten.** 1994. Meiosis-induced double strand break sites determined by yeast chromatin structure. Science **263:**515–518.

52. **Xu, L., B. M. Weiner, and N. Kleckner.** 1997. Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. **11:**106–118.
53. **Yamagata, K., J. Kato, A. Shimamoto, M. Goto, Y. Furuichi, and H. Ikeda.** 1998. Bloom's and Werner's syndrome genes suppress hyperrecombination in yeast *sgs1* mutant: implication for genomic instability in human diseases. Proc. Natl. Acad. Sci. USA **95:**8733–8738.
54. **Yu, C.-E., J. Oshima, Y.-H. Fu, E. M. Wijsman, F. Hisama, R. Alisch, S. Matthews, J. Nakura, T. Miki, S. Ouais, G. M. Martin, J. Mulligan, and G. D. Schellenberg.** 1996. Positional cloning of the Werner's syndrome gene. Science **272:**258–262.