Published in final edited form as:
DNA Repair (Amst). 2008 April 2; 7(4): 558–571.

The genetic consequences of ablating helicase activity and the Top3 interaction domain of Sgs1

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Abstract

Sgs1, the RecQ helicase homolog, and Top3, the type-IA topoisomerase, physically interact and are required for genomic stability in budding yeast. Similarly, topoisomerase III genes physically pair with homologs of *SGS1* in humans that are involved in the cancer predisposition and premature aging diseases Bloom, Werner, and Rothmund-Thompson syndromes. In the absence of Top1 activity, sgs1 mutants are severely growth impaired. Here, we investigate the role of Sgs1 helicase activity and its N-terminal Top3 interaction domain by using an allele replacement technique to integrate mutant alleles at the native *SGS1* genomic locus. We compare the phenotype of helicase-defective (*sgs1-hd*) and N-terminal deletion (*sgs1-N*Δ) strains to wild-type and *sgs1* null strains. Like the *sgs1* null, *sgs1-hd* mutations suppress *top3* slow growth, cause a growth defect in the absence of Srs2 helicase, and impair meiosis. However, for recombination and the synthetic interaction with *top1Δ* mutations, loss of helicase activity exhibits a less severe phenotype than the null. Interestingly, deletion of the Top3 interaction domain of Sgs1 causes a *top3*-like phenotype, and furthermore, this effect is dependent on helicase activity. These results suggest that the protein-protein interaction between these two DNA-metabolism enzymes, even in the absence of helicase activity, is important for their function in catalyzing specific changes in DNA topology.

Keywords

Sgs1; Top3; helicase; topoisomerase; recombination; Saccharomyces cerevisiae

1. Introduction

The *S. cerevisiae SGS1* gene belongs to a family of DNA helicases first defined by the *E. coli* RecQ helicase [1]. All RecQ homologs studied to date are important for genomic integrity (reviewed in [2,3]). In humans, mutations in three of the five known RecQ/Sgs1 homologs are involved in tumor suppression and disease syndromes: Bloom syndrome (BS) and Werner Syndrome (WS), caused by mutations in the BLM and WRN genes, respectively, and three syndromes - Rothmund Thompson (RTS), RAPADALINO, and Baller-Gerold (BGS), caused by mutations in the RECQ4 gene [4-8]. These syndromes variously display features of premature aging, cancer predisposition, developmental abnormalities, and genomic instability (for recent review see [9])

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The Sgs1 helicase was discovered by both genetic and physical interactions with Top3, a prokaryotic-like type-I topoisomerase [10]. Strains mutant for *top3* have a pleiotropic phenotype including a severe growth defect caused by a cell-cycle delay in late S/G_2, hyper-recombination at multiple loci, increased chromosome nondisjunction and sensitivity to the DNA damaging agents MMS and HU, meiotic defects, and an impaired intra S-phase checkpoint [10-15]. The phenotype of *sgs1* mutants resembles that of *top3* mutants but in each case the *sgs1* defects are less severe and, for the most part, *sgs1* is epistatic to *top3* [10,16, 17]. For example, mutation of *sgs1* suppresses the slow growth of *top3* mutants to the rate of an *sgs1* mutant.

Recently, Rmi1, a third member of the Sgs1-Top3 complex, was discovered [18,19]. Biochemical studies suggest that the Sgs1-Top3-Rmi1 complex plays a role in processing HR intermediates, restarting failed replication forks, and activating S-phase checkpoint arrest [17-25]. Rmi1 may promote binding specifically to branched DNA structures and/or stimulate Top3 strand passage [28,29]. In humans, a conserved complex of the homologous BLM-hTOPO IIIα-BLAP75/RMI1 functions in similar processes [26-30].

These functional roles for Sgs1-Top3-Rmi1 in yeast are evidenced by synthetic sickness or lethality with other genes involved in replication and recombination, such as *srs2, rrm3, slx1, slx4, mus81, mms4 and top1* [12,19,31-36,40]. These synthetic interactions, along with many of the defects in *sgs1* and *top3* mutant cells are likely a result of toxic or unresolved HR events, since they can be suppressed by mutation of genes involved in the early steps of HR such as *RAD51, RAD52, RAD54, RAD55, and RAD57* [18,19,21,37-39].

The RecQ, Blm, Wrn, and Sgs1 proteins all possess enzymatic helicase activity with a 3′ to 5′ polarity [1,40,42,43]. Yet the signature helicase domain of the eukaryotic genes only covers about a third of the length of the respective proteins, approximately 400 amino acids (AA) (Fig. 1A). For example, helicase activity has been demonstrated for Sgs1 in a fragment from AA 400-1268, indicating that much of the protein is dispensable for helicase function [44]. Furthermore, loss of the enzymatic helicase activity of Sgs1 is responsible for some, but not all aspects of the *sgs1* mutant phenotype including chromosome loss and missegregation as well as synthetic lethality with *srs2*Δ [47].

Sgs1 interacts physically with Top3, Top2, Rad16, Rad51 and Rmi1 as well as other proteins [10,13,16,18,19,48]. Genetic and physical evidence suggest that the interaction between eukaryotic RecQ and topoisomerase III homologs is of central importance for the function of both proteins in DNA metabolism. In humans, the BLM interacts with hTOPO IIIα and stimulates its strand-passage activity, and, together with RMI1, this complex can function as a double Holliday junction (dHJ) dissolvase [28,29,49,50]. In yeast, fusion of Sgs1 and Top3 into a single peptide complements several aspects of the *sgs1* phenotype [51]. The Sgs1-Top3 interaction is important in the absence of *srs2* and *top1*, as well as for complementation of MMS sensitivity and suppression of HR [46,52].

Here we investigate the roles of Sgs1 helicase activity and the Sgs1 interaction with Top3 by analyzing mutant alleles that disrupt these functions. In contrast to many previous studies, we only studied alleles integrated at their native genomic loci [53-55]. This permits an assessment of the true mutant phenotype and eliminates problems associated with plasmid-based complementation studies as well as position effects that may occur as a result of integration at non-native sites. We find that point mutations that inactivate the Sgs1 helicase result in many, but not all of the defects seen in *sgs1* null mutants. For example, helicase defective mutants and the null mutant all exhibit the same *top3* slow growth suppression as well as synthetic interaction with *srs2*. However, helicase mutants do not parallel the null allele for their effects on recombination at the *SUP4*-**o** locus or their growth defects in combination with *top1*

mutations. To investigate what other function of the Sgs1 protein may be responsible for these differences, we focused on the Sgs1 interaction with Top3 by deleting a region of the Sgs1 N terminus necessary for physical interaction with Top3. The phenotype of this deletion mimics the loss of Top3 when helicase activity is intact. However, when both the Top3 interaction domain and helicase activity are eliminated, an *sgs1* null phenotype results. These data add support to the notion that Sgs1 acts upstream of Top3 in DNA metabolism, and its helicase activity creates an intermediate DNA structure that requires the recruitment of Top3 for efficient resolution. Furthermore, these observations suggest that the physical interaction between the two proteins is required to coordinate their activities.

2. Materials and Methods

2.1 S. cerevisiae *strains and genetic methods*

The W1588 segregants of W303-1A (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 ura3-1 trp1-1 RAD5*) and their derivatives used are listed in Table 1 [56,57]. The crosses, growth, and transformation of strains were performed by standard methods [58]. Media were prepared as described [59], except twice the amount of leucine was used. Sporulation medium was prepared as described [60]. Standard procedures were used for mating, sporulation, and dissection [59]. Cells were grown at 30°C.

The introduction of the *K706R* mutation into *SGS1* was as described previously [53]. The plasmid-based *sgs1-K706A* allele (pJL37) was a gift from S. Brill [40]. A 4.1kb fragment (*Drd*I to *Sac*II) containing the *sgs1-K706A* allele was used to replace the genomic *sgs1*Δ*URA* disruption in W1956-1D. 5-Fluoro-orotic acid (5-FOA) resistant colonies from this transformation were confirmed to contain the entire *sgs1-K706A* ORF by PCR and restriction digestion analysis. Segregation of the alleles after genetic crosses were scored by colony PCR of individual spores using primers SGS1aa541-F and SGS1aa890-R followed by restriction digest (using *Bgl*II for *sgs1-K706R* and *Nhe*I for *sgs1-K706A*).

The *sgs1-NΔ82* allele was created by PCR amplification using Expand hi-fidelity polymerase (Roche Diagnostics) of two overlapping DNA fragments that were then fused by a third PCR reaction. Primers used in this study are listed in Table 2. One fragment contained 503bp directly upstream of *SGS1* ending at the ATG (primers -500-F and SGS1aa1-R). The second fragment overlapped the 3′ 20 bases of the first fragment and contained 1145bp of the *SGS1* ORF with the first 82 residues deleted (primers PROMOTER/aa83-F and SGS1-376-R). The 5′ primer contained this deletion by fusing 20 bases of the promoter to the ATG previously at residue 83. Fusion of the 503bp and 1145bp fragments by amplification with the outside primers (-500-F and SGS1-376R) yields a 1382bp fragment beginning 500bp upstream of the ATG and ending at amino-acid 376 of (wild-type) *SGS1* that lacks the first 82 residues. This fusion fragment was digested with *Xho*I and *Age*I and subcloned into the same sites of pWJ692 (pRS424-*SGS1*) to yield pWJ912 (pRS424-*sgs1-NΔ82*). The fragment was also digested with *Xho*I and *Aat*II and subcloned into the same sites of pWJ795 (pRS415-*sgs1-K706R*) to create pWJ913 (pRS415-*sgs1-NΔ82,K706R*). A 4.5 kb *Xho*I/*Bst*BI fragment containing the entire sgs1 ORF in addition to 206bp upstream and 202bp downstream was then used to replace the genomic *sgs1*Δ*URA3* allele of W1956-1D by cotransformation followed by replica plating to 5-FOA. Colonies that grew on 5-FOA were confirmed to contain *sgs1-NΔ82* (strain J734) and *sgs1-NΔ82,K706R* (strain J735) by PCR, restriction digests, and DNA sequencing. Sequence analysis of the entire region that had been amplified by PCR (up to bp1128 of wt *SGS1*) determined that no changes to the amino-acid sequence of Sgs1-NΔ and Sgs1-NΔ,K706R were introduced by PCR mutations.

We estimated Sgs1 protein levels in the various constructs used in this study by fusing each to a triple-HA epitope (see below). The introduction of the HA-tag did not significantly alter the

function of the proteins as measured by testing each construct for growth in combination with *top1* and *top3* mutants and by comparing their sensitivities to HU and MMS (as singles or double mutants or both, data not shown). These results indicate that the tagged alleles are representative of the untagged version. However, at the same time, all experiments described in this study were performed with untagged alleles to avoid unforeseen phenotypic complications that might be caused by the presence of the HA-epitope.

Strains J739, J740, J741, J742 and J743, which contain fusions of a triple-HA tag to the C terminus of wild-type *SGS1, sgs1-K706R, sgs1-K706A, sgs1-NΔ82, and sgs1-NΔ82,K706R*, respectively, were created by the allele-replacement method as described previously [53]. Briefly, an in-frame fusion of a triple-HA repeat to the C terminus of *SGS1* on plasmid pWJ691 was used as template for PCR amplification of a fragment of the C terminus, (bp 4110 of the *SGS1* ORF through 187 bp downstream). This HA-containing fragment was then fused by PCR to two overlapping fragments of the *K. lactis URA3* gene. Cotransformation of the two fragments and selection on medium lacking uracil yielded integrants containing the reconstructed *K. lactis URA3* gene surrounded by a direct repeat of the triple-HA epitope at the genomic locus of *SGS1* (or *sgs1-K706R*, etc.) Direct-repeat recombination events that excised the *URA3* gene were selected on 5-FOA, leaving a single copy of the triple-HA fusion. The HA-tagged alleles were verified by PCR analysis, phenotype and protein blots (see Fig. 1B).

2.2 Protein blot analysis

Strains bearing the HA-tagged alleles (J739, J740, J741, J742 and J743) were grown overnight in YPD. Protein extraction was carried out at 4°C using chilled solutions. Approximately 5×10^8 cells were collected and washed once in 1ml PBS, followed by resuspension in 500μl 25% TCA. Cells were pelleted at 4000 RPM and washed 2 times in 90% acetone before resuspension in 100μl 1% SDS with 1X protease inhibitor cocktail (Boehringer Mannheim). Cell pellets were lysed by vortexing with glass beads before addition of 5X loading buffer. Samples were then boiled for 3 minutes followed by a brief centrifugation and electrophoresis on 5% Ready Gels (BioRad). Proteins were then electroblotted to Immobilion-P membranes (Millipore) followed by blocking with 5% nonfat dry milk and blots were probed with 1:10,000 dilution of anti-HA antibody (Santa Cruz Biotechnology). ECL Plus (Amersham Pharmacia Biotech) detection reagents were used according to product protocols. Densitometry of bands from scanned films was performed using ImageQuant (Molecular Dynamics). As shown in Fig. 1B, the mutant Sgs1 proteins were present at the same levels as wild-type Sgs1, indicating no significant change in the steady state levels of the mutant proteins.

2.3 Recombination assays

A replica-plating assay for detection of recombination events causing the deletion of the *SUP4*-**o** gene was performed as previously described [11,61]. Briefly, various *sgs1* alleles were introduced into the W1868 background (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 SUP4*-**o***::URA3 RAD5*) by standard genetic crosses. At least 3 independent segregants of each genotype were assayed for recombination events of the δ-repeats surrounding the *SUP4*-**o** locus. Deletions of *SUP4*-**o** result in red (*ade2*-) colonies that require uracil for growth and are canavanine resistant.

2.4 Growth rates

Cultures were grown overnight in 5ml YPD cultures, from which between 300μl and 1ml were used to inoculate 50ml YPD cultures to similar OD_{600}densities as measured by spectrophotometer. To avoid spontaneous suppressors, at least 3 independent segregants of each genotype were assayed after fresh dissection of heterozygous diploids. The OD_{600} of each culture was measured at hourly intervals for a total of 8 to 12 hours. The negative log of these

numbers was then plotted. The growth rates were calculated from determining the slope of the straight-line portion of each graph, which defines the period of logarithmic growth after recovery from lag phase of the overnight culture before the cells reach stationary phase again.

2.5 HU and MMS sensitivities

Cells in mid-log phase cultures were counted and 10-fold dilutions from 10^5 to 10 cells per 10μl were made in YPD. 5μl of each was spotted on YPD plates and YPD plates containing HU or MMS. The plates were incubated at 30°C for 3-4 days. At least 2 segregants of each genotype were assayed twice and photographed.

2.6 Two-hybrid assays

The 2-hybrid strains used were PJ69 *MAT***a** and *MAT*α and the plasmids used were pGBD-C2 and pGAD-C2 [62]. pGBD and pGAD fusions with *TOP3* were constructed by amplification of the *TOP3* ORF with primers Top3/*Bam*H1f and Top3/*Bam*H1r followed by digestion with *Bam*HI and cloning into the *Bam*HI site of pGBD-C2. Also tested (but not shown) was the pGBT9-*TOP3* plasmid described in [10]. Both pGBD-Top3 fusions gave the same results. pWJ982 (pGAD-sgs1 AA 1 to 795) and pWJ983 (pGAD-sgs1 AA 83 to 795) were made by cloning a PCR fragment of *SGS1* into the *Sma*I and *Bgl*II sites of pGAD. The primers used are GAD-SGS1.1-5′ (pWJ982) or GAD-SGS1.83-5′ (pWJ983) and GAD-SGS1.795-R. The presence of fusion peptides was confirmed by protein blot analysis of the two hybrid strains (diploids listed below) using antibodies specific to the GAL-4 DNA binding domain and activation domain (Santa Cruz Biotechnology, data not shown.)

2.7 Cell cycle distribution

4′,6-Diamidino-2-phenylindole (DAPI) staining was performed to visualize DNA as described in [63]. YPD liquid cultures of the different strains were grown to mid-log phase before fixing in 70% ethanol and washing in water. At least 500 cells of each genotype were counted and divided into categories by morphology, as indicated in Fig. 3B.

3. Results

3.1 Mutant sgs1 *alleles integrated at the genomic locus*

A number of studies report the effect of various *sgs1* mutations on different aspects of its phenotype. In most of these reports, alleles expressed from plasmids or integrated at non-native loci were used to study complementation [40,41,45,46,52,64-68]. To avoid the ambiguities inherent in interpreting growth or lack of growth in cells transformed with a plasmid as well as to avoid potential position effects for loci integrated elsewhere, all mutant alleles used in this study were integrated at the *SGS1* genomic locus (Table 1) [53,54]. As described in the Materials and Methods, all alleles created for this study were confirmed by restriction digestion, sequence analysis and protein blot (Fig. 1B).

We analyzed the role of two major functions of the *SGS1* gene in the *sgs1* mutant phenotype--its helicase activity and its Top3 interaction. To understand the importance of the helicase activity, we studied *sgs1* alleles that contain mutations of the invariant lysine in the ATPase domain known to abrogate biochemical helicase activity (*sgs1-K706R* and *sgs1-K706A* [40, 53]). In all assays described below, both *sgs1-K706R* and *sgs1-K706A* alleles behaved identically and we refer to them together as *sgs1-hd*, for helicase-defective. Biochemical and 2-hybrid data identified the Top3 interaction region of Sgs1 to N-terminal residues within the first 100 amino acids of Sgs1 [10,41,46,52,64]. We created an *sgs1* allele encoding an N-terminal deletion protein by fusing the Sgs1 promoter to the ATG at residue 83 (*sgs1-NΔ82*). This 82 amino acid deletion eliminates the protein interaction with Top3 in 2-hybrid

experiments (data not shown). This truncation was also combined with helicase-mutant alleles of *SGS1*.

3.2 sgs1-hd *alleles exhibit synthetic interactions with mutations in other DNA metabolism genes*

To compare the phenotype of *sgs1-hd* and *sgs1* null alleles in combination with other genes, single and double mutant strains were constructed as described below. Since mutations in *SGS1* were first discovered as suppressors of *top3*Δ slow growth, we analyzed suppression of the growth defect by measuring the doubling times of at least 3 independent spores of various mutant genotypes. Both *sgs1-hd* and *sgs1*Δ suppress *top3*Δ slow growth to the same extent (Fig. 2A). The slow growth of cells lacking *top3* is largely the result of a delay in the late S/G_2 phase of the cell cycle, and *top3*Δ cells are greatly enlarged with a majority of mid-log-phase cells as large budded cells with a single nucleus in the bud neck [10,12,69] (Fig. 3A). We examined cell morphology and cell cycle distribution of the mutant strains by staining mid-log phase cells with DAPI, and similar to that observed for growth suppression, helicase mutations suppress the enlarged morphology and cell-cycle delay of *top3*Δ cells to the same degree as the null allele (Fig. 3B and data not shown).

We next examined the synthetic growth interaction between *sgs1* mutants and *top1*Δ. Fig. 2B illustrates the synergistic growth defect seen in *sgs1*Δ *top1*Δ double mutants [40]. The *sgs1-hd top1*Δ strains exhibit an intermediate doubling time between *SGS1 top1*Δ and *sgs1*Δ *top1*Δ strains. This difference suggests that loss of helicase activity is only partly responsible for the synergistic slow growth and that an additional non-helicase function of Sgs1 plays a role in growth in the absence of *top1*.

Loss of *sgs1* function also causes a severe synthetic growth defect that often results in inviability with mutations in *srs2* (Fig. 4A and [31]). This defect is alleviated by mutation of genes required for efficient HR such as *RAD51, RAD52, RAD55*, or *RAD57* [21,37,70]. We confirmed that both *sgs1*-Δ *srs2*Δ and *sgs1-hd srs2*Δ double mutant segregants are inviable or form microcolonies (see tetrad dissections in Fig. 4A and B) [47]. Furthermore, homologous recombination also contributes to this phenotype, since the *sgs1-hd srs2*Δ growth defect is suppressed by mutation of *rad51, 55* or *57* (Fig. 4C and data not shown).

3.3 Mutation of the Sgs1 helicase impairs recombination and meiosis

Mutations in *sgs1* increase mitotic recombination at numerous genetic loci including the *SUP4*-**o** locus surrounded by δ-repeats [10,12,16,69,74]. Deletion of *SUP4*-**o** occurs by recombination events between these repeats. To examine the importance of the helicase function in the suppression of mitotic recombination, we measured marker loss of *SUP4*-**o**. As shown in Table 3, deletion of *sgs1* results in a 39-fold increase in recombination. Mutation of the *sgs1* helicase, however, increases recombination frequency to only half that of the null (19-fold).

Sgs1 is thought to prevent aberrant crossing-over events during meiosis by suppressing formation of joint molecules [72]. Diploids homozygous for an *sgs1* deletion exhibit a meiotic defect that results in decreased sporulation efficiency and spore viability at least partially as a result of missegregation leading to aneuploidy [12,16,17,65,71]. To investigate the helicase function on meiosis, we examined the effect of *sgs1-hd* alleles on sporulation and spore viability. As shown in Table 4, the *sgs1-hd* alleles are recessive, since heterozygous *sgs1-hd/SGS1* diploids undergo normal meiosis. However, in homozygous *sgs1-hd* diploids, like the null, only ~20% of the cells sporulate after 3 days and show ~70% spore viability (Table 4), demonstrating that the meiotic defect is caused solely by loss of the helicase activity.

3.4 Deletion of the Top3-interaction domain of Sgs1 causes a top3-like phenotype

We next analyzed the effect of removing the Top3-interaction domain from a helicase-intact *SGS1* gene (*sgs1-N*Δ). As shown in Fig. 2A, deletion of the N-terminal 82 amino acids of Sgs1 slows growth nearly as much as deletion of *TOP3*. Similarly, *sgs1-N*Δ cells are enlarged relative to wild-type though not as much as *top3*Δ mutant cells (Fig. 3A). Indeed, the cell-cycle distribution of *sgs1-N*Δ cells is similar to *top3* mutant cells, where about 60% of cells are present as large, budded cells with a single nucleus (Fig. 3B).

Furthermore, we looked at various genetic interactions in *sgs1-N*Δ strains and found that the *sgs1-N*Δ *top3*Δ double mutant grows more slowly than either single mutant (Fig. 2A). In addition, *sgs1-N*Δ *top1*Δ doubles grow as poorly as *sgs1*Δ *top1*Δ double mutants (Fig. 2B). Finally, the genetic interaction between *sgs1-N*Δ and *srs2* resembles that seen for *sgs1*Δ and *srs2*Δ and is also suppressed by blocking recombination (Fig. 4A, D and F).

Next, we examined the interaction of the *sgs1-N*Δ allele with genes involved in the DNA damage response, which are required for reversible DNA damage-dependent cell cycle arrest (for review see [76]). Crosses to the S-phase checkpoint mutant, *mec1*Δ, and a downstream kinase, *dun1*Δ, demonstrate that loss of *top3*, but not *sgs1*Δ or *sgs1-hd*, causes a synthetic growth defect or inviability (Table 5, [14]). However, when *sgs1-N*Δ is introduced into *mec1*Δ and *dun1*Δ mutant strains, a synthetic defect identical to that of *top3*Δ is seen.

Similarly, the recombination frequency at the *SUP4*-**o** locus in *sgs1-N*Δ strains also approximates that seen in *top3* strains (Table 3 and [11,39]). These results are consistent with previous observations of elevated recombination in strains bearing alleles of *sgs1* lacking the N terminus [45,75]. Furthermore, as shown in Table 4, diploids homozygous for *sgs1-N*Δ have a meiotic defect identical to that of *top3*Δ mutants and fail to sporulate at significant levels [11,12,16,17]. However, unlike heterozygous *top3*Δ/*TOP3*, *sgs1*Δ/*SGS1* or *sgs1-hd/SGS1* diploids, *sgs1-N*Δ has a dominant negative effect on meiosis resulting in decreased sporulation ($p < 0.02$) and exhibits slightly lowered spore viability.

3.5 The top3-like phenotype of sgs1-NΔ depends on its helicase activity

As described above, loss of *sgs1* helicase activity suppresses most *top3* defects. Since *sgs1-N*Δ resembles *top3*Δ, we mutated the helicase motif of this allele (*sgs1-N*Δ,*hd*) to measure its effect in all of the assays described above. Similar to the suppression of *top3* by *sgs1-hd*, eliminating helicase activity from the *sgs1-N*Δ allele results in the equivalent of an *sgs1* null phenotype (see Table 6 for summary). Specifically, the growth defect (Fig. 2), altered cell morphology (Fig. 3A), cell cycle defects (Fig. 3B), hyper-recombination (Table 3) and synthetic defect with *mec1* and *dun1* mutants (Table 5) are suppressed. There is one case, however, where mutation of the helicase in *sgs1-N*Δ does not change the phenotype *sgs1-N*Δ. In combination with a *top1* mutation, *sgs1-N*Δ and *sgs1-N*Δ,*hd* exhibit the same growth rate as an *sgs1*Δ *top1*Δ double mutant (see Fig. 2B).

3.5 The sgs1 alleles have differential effects on sensitivity to HU and MMS

Lastly, we examined mutant *sgs1* strains for their sensitivity to hydroxyurea (HU), which stalls DNA synthesis [77] and methylmethane sulfonate (MMS), a DNA alkylating agent likely to interefere with replication fork progression [78,79]. Previous studies have shown that plasmid-based helicase-defective *sgs1* alleles fail to complement *sgs1*Δ for MMS and HU [20,45,65]. Several reports, however, showed that helicase-defective genomic *sgs1* alleles are more sensitive than a null allele to both chemicals [13,47], suggesting that presence of the non-functional protein is interfering with DNA damage repair. We confirmed that *sgs1-hd* integrated at its normal locus exhibits increased HU and MMS sensitivity (Fig. 5). Since *sgs1-N*Δ, *sgs1-N*Δ,*hd* and *sgs1* null strains are all equally sensitive to HU (Fig. 5), removal of the

N terminus, regardless of helicase function, causes HU sensitivity equivalent to deleting the entire protein. Surprisingly, *sgs1-N*Δ and *sgs1-N*Δ,*hd* strains, like the *sgs1-hd* mutant, are both more sensitive to MMS than the null (Fig. 5). This is the only case we found where an *sgs1-N*Δ,*hd* strain does not exhibit a null phenotype.

4. Discussion

4.1 Model for the function of Sgs1 helicase activity and Top3 interaction

Here, using a variety of assays summarized in Table 6, we compare the phenotypic effects of mutations that remove the enzymatic helicase and Top3-interaction functions of Sgs1. All constructs used in this study are under control of the wild-type *SGS1* promoter and expressed from the native genomic *SGS1* locus. We expand on previous studies to show that point mutation of the *SGS1* helicase resembles an *sgs1* null allele for suppression of *top3* slow growth, for meiotic defects and for synthetic lethality with *srs2* mutations. Similarly, synthetic lethal interactions between *sgs1* and *slx1, mms4, slx3, slx4, slx5*, and *slx8* are also dependent on Sgs1 helicase activity [34]. However, some of the defects caused by loss of helicase activity are less severe than *sgs1* null defects as measured by both growth in a *top1* mutant background and recombination at the *SUP4*-**o** locus (Fig. 2B, Table 3). For these two assays, the null phenotype is seen only when the Top3 interaction is eliminated from a helicase-deficient protein (*sgs1-N*Δ,*hd*). These data suggest that the presence of the Sgs1-Top3 interaction serves some function even in the absence of Sgs1 helicase activity. Moreover, removing the interaction domain from the wild-type helicase active *SGS1* allele (*sgs1-NΔ*) causes a phenotype that is even more severe than an *sgs1* null and is similar to a *top3* mutant. Thus, a *top3*-like phenotype likely results from preventing Top3 from interacting with a catalytically active Sgs1.

We note that in several complementation studies, plasmid-borne *sgs1-hd* alleles were unable to suppress the HR defect of *sgs1Δ* [65,80] but behaved like wild-type *SGS1* in growth assays of *top3Δ sgs1Δ* and *top1Δ sgs1Δ* strains and in its ability to rescue the meiotic defects of homozygous diploid *sgs1* mutants [40,45,46,65]. Also, recent studies of the meiotic function of Sgs1 utilized N-terminal fragments of the protein (aa 1-795) entirely lacking the helicase domain since this allele improved sporulation efficiency relative to a null allele [72,73]. It has been suggested that conflicting observations of the *sgs1* mutant phenotype could result from differential expression from plasmid vectors [47]. Such dosage effects, and/or strain differences, may account for these discrepancies. For example, the MR strain used in several of these studies differs from the W303 genetic background used here and in many other studies. In MR, *sgs1Δ* has a significantly different meiotic defect and shows incomplete epistasis to *top3Δ*. In addition *top3Δ* is “semi-lethal” in MR strains. [65,67].

The data presented here are consistent with the original proposition that the Top3 phenotype results largely from unresolved recombinogenic substrates created by Sgs1 activity [10,17]. A simplified model that both incorporates these data and includes an alternative pathway that utilizes Top1 is presented in Fig. 6A. In this schematic, a DNA substrate, such as a collapsed or stalled replication fork, is processed to an intermediate, (1), that is converted by the Sgs1 helicase to an intermediate, (2), that is toxic if unresolved into a final product, (3), by Top3. A primary candidate for one of the substrates of the Sgs1-Top3-Rmi1 complex is the Holliday junction (HJ), a key intermediate in recombinational repair and recovery from replication fork damage (see [81,82] for review.) RecQ, BLM, WRN, RECQL1, RECQ5β, and Sgs1 are all able to bind and efficiently unwind HJ structures *in vitro* [83-88]. RECQL1, BLM and WRN efficiently promote ATP-dependent HJ branch migration, and, in the case of human and *Drosophila* BLM/TopIII complexes, ATP-dependent HJ dissolution [28,85,86,89-91]. Alternatively, this intermediate may be a regressed replication fork [25,47,76].

Our model is consistent with the notion that resolution of HJs by a RecQ-Top3 mechanism is a two-step process: ATP-dependent HJ branch migration by a RecQ helicase to form a hemicatenane (Fig. 6A, intermediate 2), immediately followed by decatenation by a type IA topoisomerase via its strand passage activity (Fig. 6A, final product 3). As suggested by others [25,92], the observation that MMS induced X-molecules persist in *sgs1*, *sgs1 top3*, and *top3* cells supports the notion that, in *sgs1* cells, these X-molecules represent dHJs (substrate 1), whereas those in *top3* cells represent hemicatenanes (intermediate 2). Furthermore, novel ternary and quaternary joint molecules and inter-sister dHJs persist in *sgs1* mutant cells during meiosis [72]. However, recent observations of Nickoloff and colleagues that crossover and gene conversion tract length may be independent of helicase function, suggest that helicase activity is not involved in HJ branch migration [47]. Thus, intermediate 2 in our model may be a different type of X-shaped intermediate, such as a regressed replication fork.

4.2 Function of the The N terminus of Sgs1

We suggest that, in the absence of Top3, the Sgs1 helicase creates DNA lesions that are the primary cause of the *top3* phenotype (intermediate 2* in Fig. 6B). Furthermore, we propose that these lesions also persist when the Sgs1-Top3 interaction is eliminated by deletion of the N terminus of Sgs1, even in the presence of a functional Top3 (2* in Fig. 6C). The similarities between the *sgs1-NΔ* and *top3Δ* mutant phenotypes are due to persistence of the same toxic lesions (Fig 6B and C), and in both cases loss of *sgs1* helicase activity prevents their formation (Fig. 6D and E). We previously showed that this toxicity is at least partly a result of the activity of HR, since *top3Δ* defects can be suppressed by mutation of *RAD51, 54, 55* and *57* and members of the Shu complex [37,39,69]. Our model predicts that mutation of the same genes would also improve the growth of *sgs1-NΔ* strains and that is indeed the case (data not shown).

Further support for the equivalence of *top3* and *sgs1-NΔ* defects comes from genetic interactions with Pif1, a 5′ to 3′ helicase involved in nuclear and mitochondrial DNA metabolism. We previously demonstrated that overexpression of the Pif1 helicase suppresses slow growth, MMS and HU sensitivity of *top3Δ*, and that loss of *pif1* is synthetically lethal with loss of *top3* but not *sgs1* [93]. Similarly, overexpression of Pif1 suppresses *sgs1-NΔ* slow growth, HU and MMS sensitivity. Moreover, *sgs1-NΔ* is synthetically lethal with *pif1* and this lethality is dependent on the *sgs1-NΔ* helicase activity [93]. Thus, Pif1 helicase activity is essential to counteract Sgs1 helicase activity and promote survival in the absence of Top3, or the absence of the Sgs1-Top3 interaction.

If the only function of the N terminus of Sgs1 were to interact with Top3, then removal of this domain should not alter the phenotype of a *top3Δ* strain. However, the growth defect of *sgs1-NΔ top3Δ* strains is greater than *top3Δ* single mutant strains (Fig. 2A). One hypothesis for this difference is that removal of the interaction frees the Sgs1 helicase to act ectopically at inappropriate sites, thereby causing a more severe phenotype. Another possibility, first suggested by Mullen and colleagues, is that the N-terminal region of Sgs1 is involved in regulating its helicase activity and that removal of this domain creates a hyperactive allele [45]. Thus, in addition to eliminating the Top3 interaction, deletion of the N terminus could alter the enzymatic activity of the helicase (e.g., processivity) leading to an increase of the toxic intermediate.

Although there is no biochemical evidence for the N-terminal deletion directly influencing Sgs1 helicase activity, some genetic data support the notion that Sgs1-NΔ contains a hyperactive helicase. The DNA damage sensitivity of the *sgs1-NΔ* alleles can be suppressed by overexpressing Top3, and overexpression of Top3 improves the growth of *sgs1-NΔ* strains ([64] and data not shown) suggesting that the increase in topoisomerase activity counteracts the toxic effects of excess helicase activity. Additionally, functional interactions are known to

regulate other RecQ helicases, such as the stimulation or attenuation of WRN and BLM helicase activities by interaction with TRF2 [94] or p53 [95], respectively.

4.3 Genetic interactions between sgs1 *and* top1

We propose that a subset of structures processed by the Sgs1-Top3 pathway can also be processed via an alternative Top1-dependent pathway (Fig. 6A). Unlike the epistatic relationship between *sgs1Δ* and *top3Δ* indicating that the two genes function in a single pathway (Fig. 2A), *sgs1*Δ and *top1*Δ exhibit a synergistic defect suggesting that these two genes function in separate but overlapping pathways (Fig. 2B and Fig. 6F). Notably, mutant *sgs1* alleles cause different effects in a *top1Δ* background than in a *top3Δ* background. Although growth of both *top3Δ* and *top1Δ* strains is slowed by the *sgs1-NΔ* allele, mutation of the *sgs1-NΔ* helicase eliminates this effect in *top3Δ* strains but not in a *top1Δ* background (Fig. 2A and 2B and Fig. 6D and 6H). One interpretation of these data is that removal of the N terminus is responsible for the *sgs1Δ top1Δ* synergistic growth defect and loss of helicase activity is unimportant. However, Sgs1 helicase activity clearly plays a role in the absence of *top1* as seen by comparing the growth of *SGS1 top1Δ* strains with *sgs1-hd top1Δ* strains (Fig. 2B and 6G). In addition, the N-terminal Top3 interaction of Sgs1 is important for growth in a *top1Δ* background regardless of helicase activity, as indicated by the growth difference between *sgs1-hd top1Δ* and *sgs1-NΔ,hd top1*Δ strains (Fig. 2B and 6G and 6H). These results are in agreement with previous studies, including observations that Sgs1 N-terminal fragments fully lacking the helicase domain improve the growth of *sgs1Δ top1Δ* strains ([41,45] and our unpublished data).

4.4 Differences in allele sensitivities to HU and MMS

The effects of the *sgs1* mutant alleles in this study exhibited slight differences for HU and MMS sensitivities. The increased sensitivity to HU seen in *sgs1-hd* is relieved by removal of the Top3 interaction (Fig. 5). Perhaps removing the interaction frees Top3 from binding catalytically inactive Sgs1, and improves the ability of Top3 to act in repairing HU damage as well as improving growth in the absence of *top1*. The observation that the *sgs1-NΔ,hd* allele has a greater MMS sensitivity than complete deletion of *SGS1* suggests that the helicase-inactive allele is interfering with the cellular response to MMS damage in a Top3-independent fashion. The inactive *sgs1-NΔ,hd* could, for example, interact with other proteins to essentially sequester them in an inactive complex. Thus, a protein fragment that is unable to interact with Top3 and is incapable of catalyzing DNA unwinding likely preserves another aspect(s) of Sgs1 function, e.g., interaction with Top2 or Rmi1 [16,18,19]. In the null allele, this interaction is missing accounting for its less severe phenotype.

The response of *sgs1* mutant alleles to HU and MMS are of interest, particularly in light of the discovery of a new class of Sgs1/Top3 suppressors known as the “Shu” genes (suppressor of *sgs1* HU sensitivity) that includes Shu1, Shu2, Csm2, and Psy3 proteins [69,96]. Mutation of any one of these genes suppresses the HU sensitivity of Top3 and Sgs1 but not their MMS sensitivity. These proteins interact in pair-wise combinations, and likely form a complex since the quadruple mutant has the same phenotype as any single or various double mutant combinations. [69,96].

In conclusion, this study elucidates the central roles of two major elements of the Sgs1 protein: its catalytic helicase activity and its Top3 interaction. Our data highlight the significance of the physical interaction of Sgs1 and Top3 in suppressing deleterious Sgs1 helicase activity. Bloom, Werner, and Rothmund-Thompson syndromes are all distinct human diseases with an increased incidence of cancers caused by mutations in the human homologs of *SGS1* and *RECQ*, and in many ways *sgs1* mutants phenocopy several of these diseases. Thus, the evolutionary conservation of the RecQ family interactions with topoisomerase III isozymes

raises the possibility that this protein-protein interaction may be important for tumor suppression in humans as well.

Acknowledgments

We thank Uffe Mortensen, Frederic Foucault, Greg Freyer, Morten Dunø, Michael Lisby, Erika Shor, Naz Erdeniz, Robert Reid, Marisa Wagner, and Lorraine Symington for helpful discussions concerning this work. We especially thank Kara Bernstein for careful reading of the manuscript, Qi Feng for her assistance in preparation of this manuscript and Ivana Sunjaveric for technical support. J.W. is particularly indebted to Shan Sockanathan, Floria Lupu, and Jonathan Eggenschweiler for providing additional materials and support throughout the course of this work. This work was supported by GM07088, a pre-doctoral training grant (J.W.) and by NIH grant GM50237 (R.R.).

Abbreviations

MMS, methyl methanesulfonate; HU, hydroxyurea; HR, homologous recombination; HJ, Holliday junction; dHJ, double Holliday junction.

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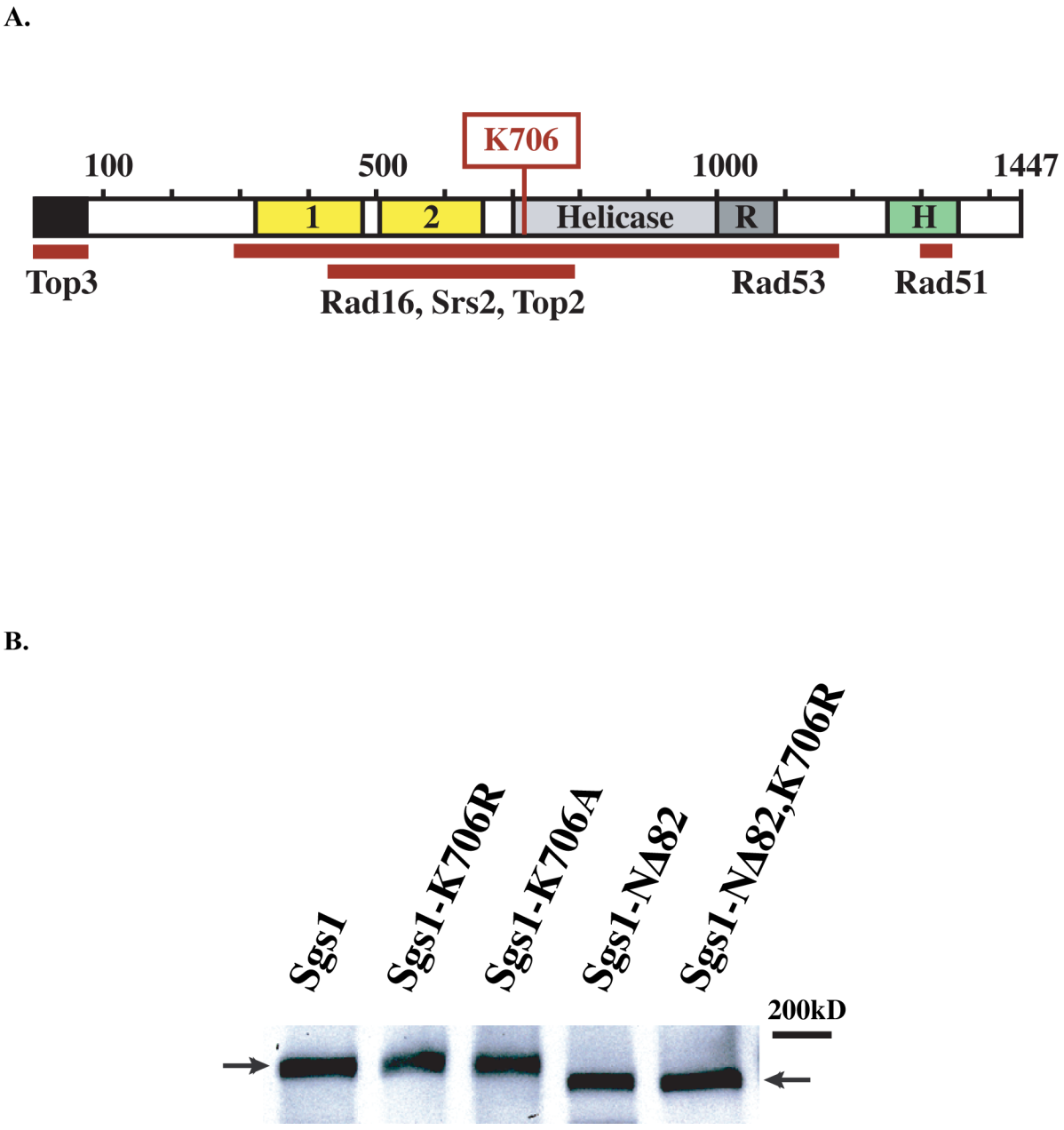


Fig. 1.

(A) Diagram of Sgs1 protein showing regions of interaction with other proteins as well as functional domains. The N-terminal 82-aa deletion is indicated in black and the Walker-A box invariant lysine at residue 706 is shown in red. Other features of the Sgs1 protein are illustrated as follows: the two acidic regions ("1" and "2") are in yellow, the helicase domain is gray, the RecQ C-terminal homology region is in dark gray, and the Helicase and RNaseD C-terminal region (HRDC) is in green. (B) Protein blot of triple-HA-tagged alleles of Sgs1. Alleles are indicated at the top. Left arrow indicates full-sized 1447aa Sgs1 protein, right arrow indicates mobility shift of N-terminal deletions at 1365aa; size marker is indicated at right. Blot was probed with 1:10,000 dilution of HA antibody (Santa Cruz Biotechnology) and visualized using

ECL Plus (Amersham Pharmacia Biotech) detection reagents according to product protocol (see Material and Methods for details).

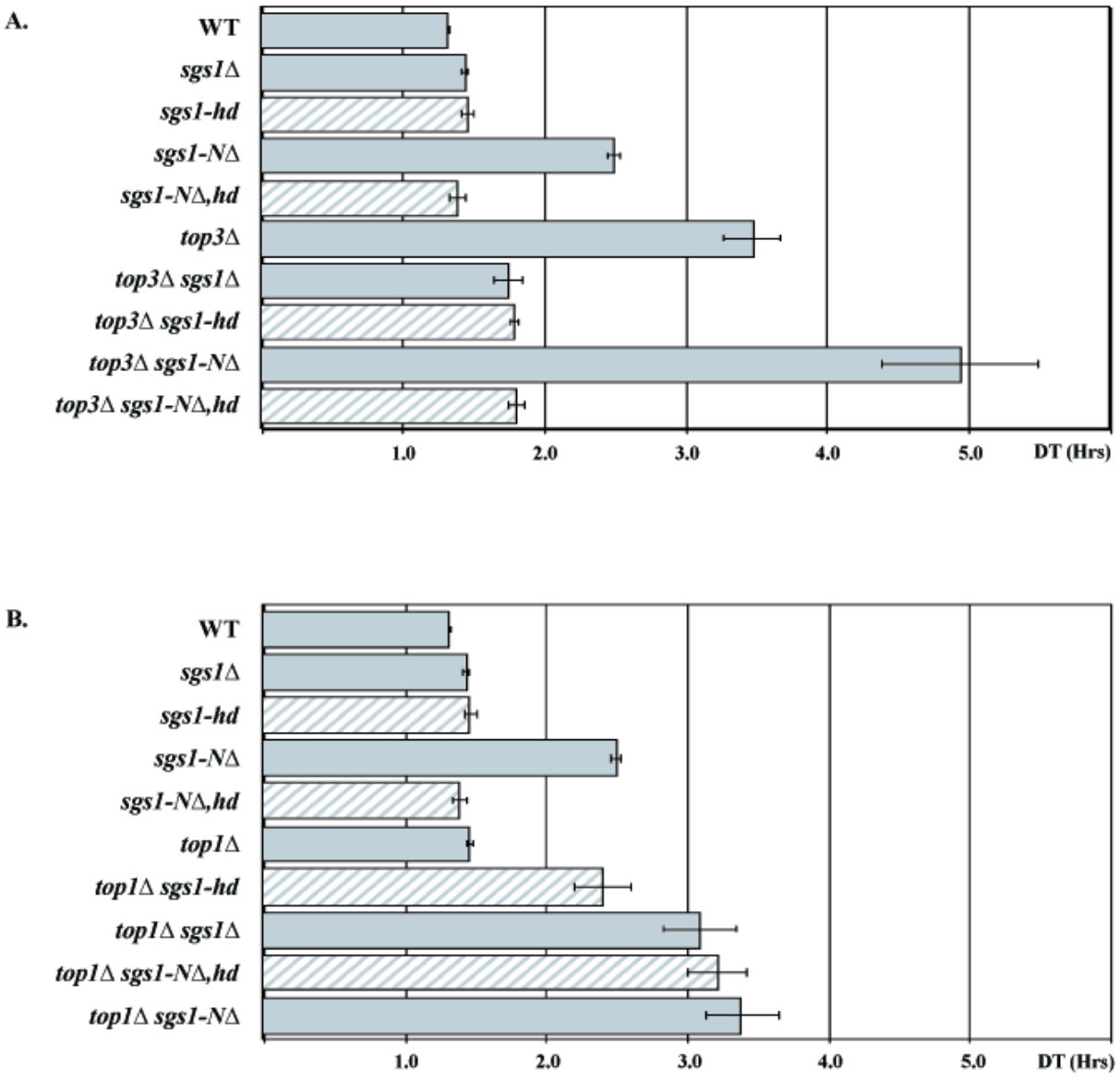
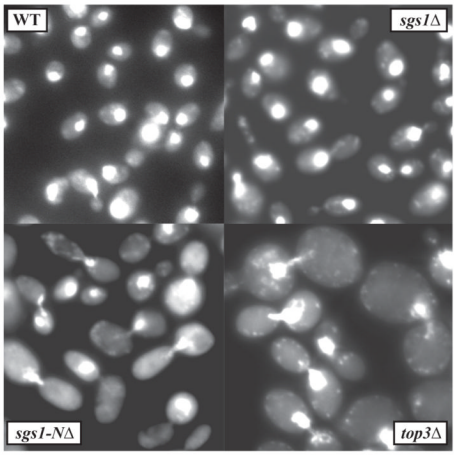


Fig. 2.

Bar graphs of doubling times (DT) of various mutant strains. Combinations of mutant strains are indicated on the left. (A) Strains mutant for *sgs1* and *top3*. (B) Strains mutant for *sgs1* and *top1*. The growth rate of logarithmic cells from each culture was calculated and is displayed. Growth rates were measured 3 times for each strain.

A.



B.

Strain	% in class			
WT	49.5	21.8	18.2	10.5
SGS1	42.4	32.9	14.7	10.0
sgs1-hd	41.0	33.5	15.9	10.6
sgs1-NΔ,hd	37.2	31.3	19.7	11.8
sgs1-NΔ	22.5	14.9	49.9	12.7
***top3*Δ**	14.5	23.4	54.4	7.7

Fig. 3.

(A) Cell cycle distribution of different mutant strains. (A) Mid log-phase cultures of WT, *sgs1*Δ, *sgs1-N*Δ and *top3* mutant strains were fixed in EtOH and stained with DAPI for visualization of DNA and photographed. (B) For the four strains shown in (A) as well as for *sgs1-N*Δ *and sgs1-hd*, at least 500 cells of each genotype were observed and classed according to morphology as shown. These classes correspond roughly to cell cycle phases G1, S, G2/M, M/G1. The percentages of each class are indicated.

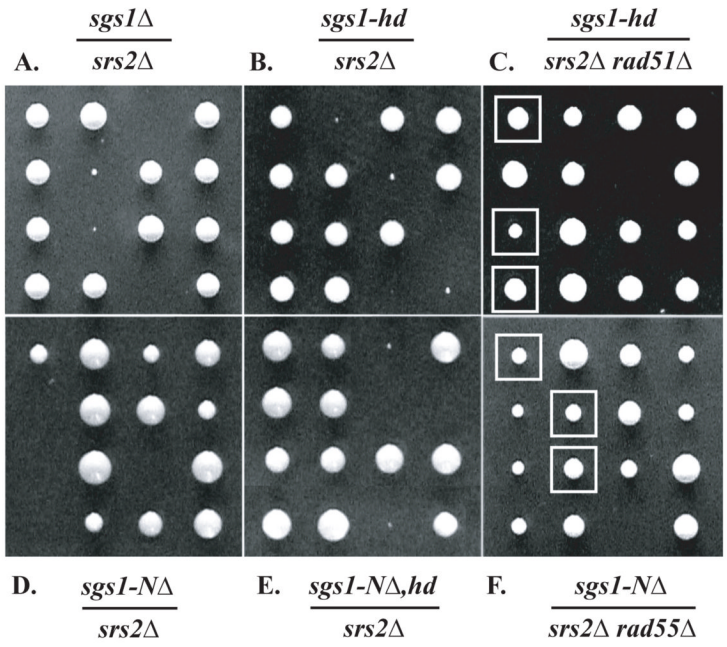


Fig. 4.

Representative tetrads to illustrate genetic interactions between *sgs1* and *srs2*. Tetrads are oriented horizontally, i.e., the third spore of the first tetrad in **A.** has not produced a colony. Genotypes of haploid parents are indicated in figure, and are: **A.** Diploid W1857, heterozygous for *sgs1::URA3* and *srs2::HIS3*. **B.** Diploid 1914, heterozygous for *sgs1-K706R* and *srs2::HIS3*. **C.** W3058, heterozygous for *sgs1-K706R*, *srs2::HIS3*, and *rad51::LEU2*. **D.** W2068, heterozygous for *sgs1-NΔ82* and *srs2::HIS3*. **E.** W2074, heterozygous for *sgs1-NΔ,hd* and *srs2::HIS3*. **F.** W2725, heterozygous for *sgs1-NΔ82*, *srs2::HIS3*, and *rad55*Δ. In **A., B., D.,** and **E.,** the colonies that are missing or are present as small colonies represent double mutants. In **C.** and **F.**, triple mutants are indicated by squares.

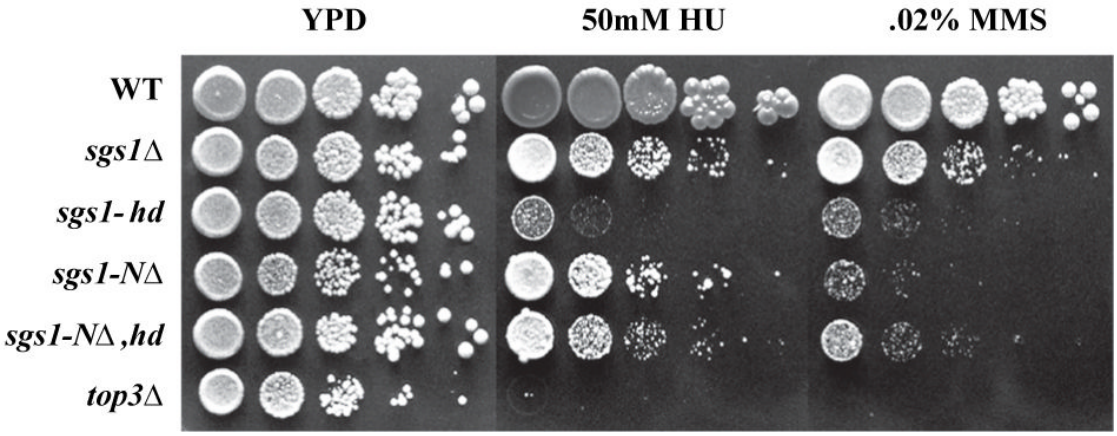


Fig. 5.

HU and MMS sensitivity of *sgs1* and *top3* mutants. Mid-log phase YPD-grown cells of mutant strains were spotted in 10-fold serial dilutions on YPD plates and YPD plates containing 50mM HU or 0.02% MMS and grown for 3 days at 30°C.

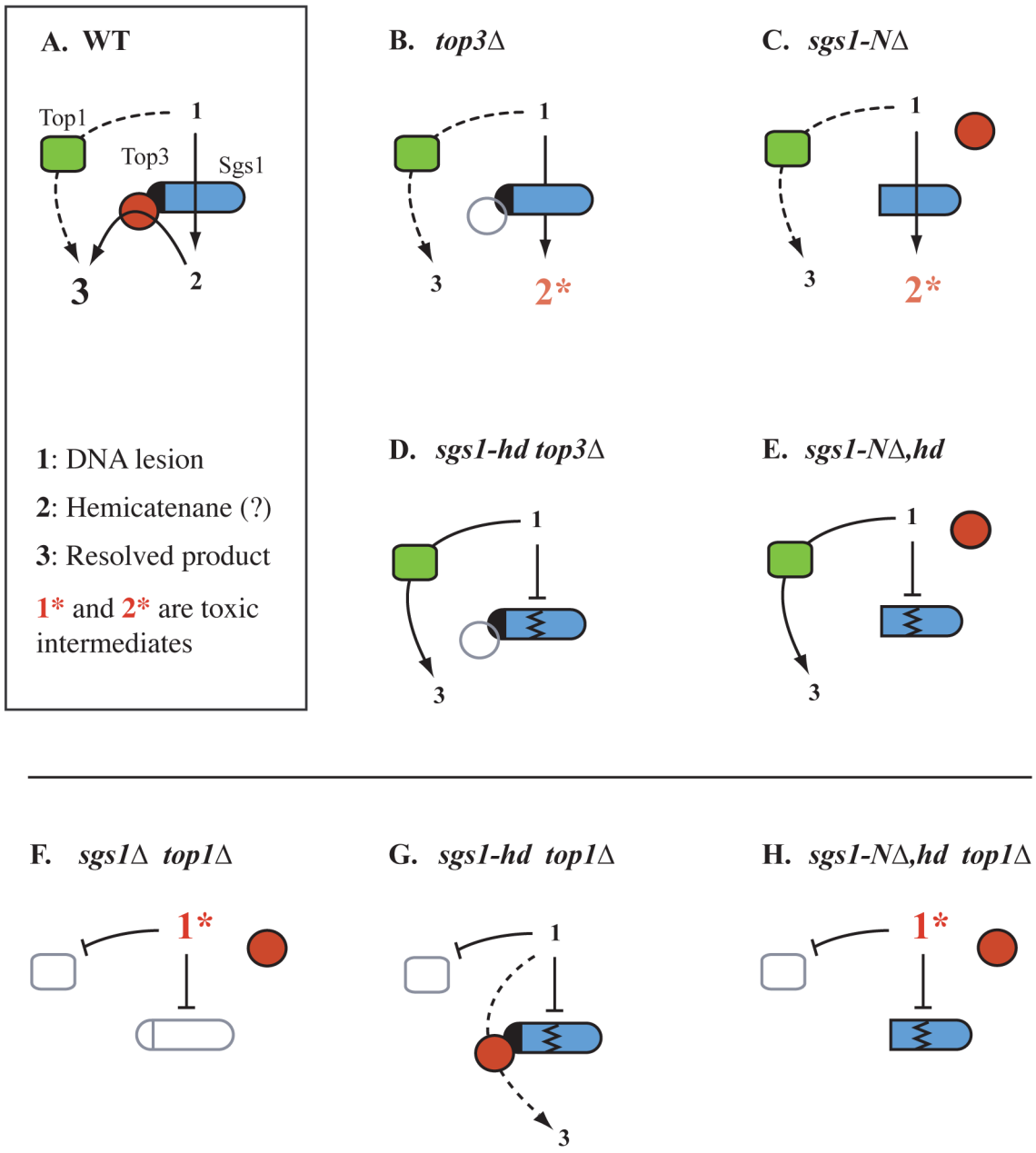


Fig. 6.

Model of relationships between Sgs1, Top3 and Top1. Genotype is indicated above each schematic. In wild-type strains (A) a DNA structure (1), e.g., a collapsed or stalled replication fork, can be converted by a series of steps not shown to structures that are acted on by Sgs1 (e.g., a double Holliday junction) and converted into an intermediate (2), e.g., a hemicatenane, which is finally resolved into the final product (3) by Top3. An alternative pathway utilizing Top1 is also shown, which "acts on" an upstream intermediate to generate a viable cell, albeit one with increased recombination and sensitivity to DNA damaging agents (indicated by the smaller size of the number "3"). According to this model, the phenotypes of *sgs1-N*Δ and *top3*Δ in (B) and (C), respectively, result from persistence of a toxic intermediate (2*).

Elimination of the helicase activity of full-length *SGS1* (D) or *sgs1-N*Δ (E) prevents formation of intermediate (2) and the alternative Top1 pathway is active. Blocking this alternative pathway (F) results in a synergistic growth defect caused by the accumulation of a different toxic intermediate (1*). In the presence of a helicase inactive Sgs1 protein, the synergistic growth defect is less severe (G). However, this improvement in growth depends on the Top3 interaction domain of Sgs1 (H) .

Table 1

Strains used in this study

Strain	Genotype
W1588-4C	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5*
W1588-4A	*MAT***α** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5*
J276	W1588-4A *sgs1-K706R*
J730	W1588-4C *sgs1-K706A*
J734	W1588-4A *sgs1-NΔ82*
J735	W1588-4A *sgs1-NΔ82,K706R*
J739	W1588-4A *SGS1-3HA*
J740	W1588-4A *sgs1-K706R-3xHA*
J741	W1588-4A *sgs1-K706A-3xHA*
J742	W1588-4A *sgs1-NΔ82-3xHA*
J743	W1588-4A *sgs1-NΔ82,K706R-3xHA*
W1874	Diploid wild-type (W1588-4C/W1588-4A)
W1956	Diploid *sgs1::URA3/SGS1 top1::HIS3/TOP1*
W1958	Diploid *sgs1::HIS3/SGS1 top3::TRP1/TOP3*
W1857	Diploid *sgs1::URA3/SGS1 srs2::HIS3/SRS2*
W1914	Diploid *sgs1-K706R/SGS1 srs2::HIS3/SRS2*
W2008	Diploid *sgs1-K706A/SGS1 srs2::HIS3/SRS2*
W2087	Diploid *sgs1-NΔ82/SGS1 srs2::HIS3/SRS2*
W2088	Diploid *sgs1-NΔ82,K706R/SGS1 srs2::HIS3/SRS2*
W1875	Diploid *sgs1::HIS3/sgs1::HIS3*
W1949	Diploid *top3::TRP1/top3::TRP1*
W1950	Diploid *sgs1-K706R/sgs1-K706R*
W2034	Diploid *sgs1-K706A/sgs1-K706A*
W1911	Diploid *sgs1-K706R/SGS1*
W2036	Diploid *sgs1-K706A/SGS1*
W2069	Diploid *sgs1-NΔ82/SGS1*
W2075	Diploid *sgs1-NΔ82,K706R/SGS1*
W2065	Diploid *sgs1-NΔ82/SGS1top1::HIS3/TOP1*
W2071	Diploid *sgs1-NΔ82,K706R/SGS1top1::HIS3/TOP1*
W2066	Diploid *sgs1-NΔ82/SGS1top3::TRP1/TOP3*
W2072	Diploid *sgs1-NΔ82,K706R/SGS1top3::TRP1/TOP3*
W2068	Diploid *sgs1-NΔ82/SGS1 srs2::HIS3/SRS2*
W2074	Diploid *sgs1-NΔ82,K706R/SGS1 srs2::HIS3/SRS2*
W1959	Diploid *sgs1::HIS3/SGS1 top3::TRP1/TOP3 SUP4-***o***::URA3*
W1934	Diploid *sgs1-K706R/SGS1 top3::TRP1/TOP3 SUP4-***o***::URA3*
W2082	Diploid *sgs1-NΔ82/SGS1 SUP4-***o***::URA3*
W2083	Diploid *sgs1-NΔ82,K706R/SGS1 SUP4-***o***::URA3*
W2089	Diploid *sgs1-NΔ82/SGS1 mec1::TRP1/MEC1 sml1-1/SML1*
W2090	Diploid *sgs1-NΔ82,K706R/SGS1 mec1::TRP1/MEC1 sml1-1/SML1*
W2094	Diploid *sml1::HIS3/SML1 mec1::TRP1/MEC1 sgs1::URA3/SGS1 top3::LEU2/TOP3*
W2706	Diploid *sgs1::URA3/SGS1 srs2::HIS3/SRS2 rad55Δ/RAD55*
W2722	Diploid top3::TRP1/TOP3 srs2::HIS3/SRS2 rad55Δ/RAD55
W2725	Diploid *sgs1-NΔ82/SGS1 srs2::HIS3/SRS2 rad55Δ/RAD55*
W2726	Diploid *sgs1-NΔ82,K706R/SGS1 srs2::HIS3/SRS2 rad55Δ/RAD55*
W3058	Diploid *sgs1-K706R/SGS1 srs2::HIS3/SRS2 rad51::LEU2/RAD51*

All strains are *RAD5* isogenic derivatives of W303 [56,57]

Table 2

Primers used in this study

Name	Sequence
SGS1-PROMOTER/aa83-F	CACGTACACACAAGGCGGTAATGCAAACTTTGTCGAAC
SGS1us500-F	GGCTCAAACTGATCAGCGTTCGG
SGSaa1-R	CATTACCGCCTTGTGTGTACG
SGS1aa376-R	GACTGGTTCTCTTGAACGC
SGS1us116-F	GCTGATATACGGATCAATAGAG
SGS1aa119-R	CGAGCCATTGGGCGTCCTCGGG
SGS1aa376-R	GACTGGTTCTCTTGAACGC
GAD-SGS1.1-5′	TAATATCCCCCGGGATGGTGACGAAGCCGTC
GAD-SGS1.83-5′	TAATATCCCCCGGGATGCAAACTTTGTCGAACG
GAD-SGS1.795-3′	AGGAAGATCTTCCTCTACTGATAGCTCTCTTGC
SGS1aa541-F	GCCGTCAACACATGCCCATGTC
SGS1aa890-R	GATTTAACCGCATCACAG
D/ds56-31r	GTGCTGCAGGTGTAAACTTGGAATGCTTGGCGAATGGTGTCG
E/1418f	GATACATCTACAACCGGCACCAAGGTCCATCAGTAAGTCGTCC
SGS1ct/NheI-F	TCAAAGGGCTAGCTTAGAGTAGAAAAATAAATAGTGTTAC
SGS1ct/NheI-R	TCAAAGGGCTAGCTCCTTTCTTCCTCTGTAGTGACCTCGG
SGSds252R	GACGCATTTATGCACATATGTAG
3HA/NheR	CGAAGCGCTAGCTATCTTTTACCCATACGATG
3HA/NheF	CGAAGCGCTAGCCTGGCCAGCGTAATCTGGAACG
SGS1HAcheck-R	GCCCGCATAGTCAGGAACATCG
Top3/BamH1f	TCGCGGATCCTAATGAAAGTGCTATGTGTCGCAGAG
Top3/BamH1r	TCGCGGATCCTTACATGGATGCCTTGACACGGTC

Table 3

Recombination frequency at *SUP4*-**o**

Strain	Frequency ($\times 10^{-4}$)	Fold increase
SGS1	1.7 ± 0.3	1
*sgs1*Δ	64 ± 6	39
sgs1-hd	31 ± 3	19
*sgs1-N*Δ	142 ± 13	86
*sgs1-N,*Δ *hd*	70 ± 9	42

Table 4

Meiotic defects as measured by sporulation and spore viability

Strain	Sporulation (%)	Spore Viability (%)
SGS1/SGS1	60	94
SGS1/sgs1-hd	58	91
sgs1Δ/sgs1Δ	22	72
sgs1-hd/sgs1-hd	21	68
sgs1-NΔ/SGS1	46	85
sgs1-NΔ/sgs1-NΔ	<3	<3
top3Δ/top3Δ	<3	<3
top3Δ/TOP3	58	91

Table 5

Viability with *mec1/dun1* mutations

Inviable/sick	Viable
mec1Δ	*mec1Δ sml1Δ*
mec1Δ sml1Δ top3Δ	*mec1Δ sml1Δ top3Δ sgs1Δ*
mec1Δ sml1Δ sgs1-NΔ	*mec1Δ sml1Δ sgs1-NΔ,HD*
dun1Δ top3Δ	*dun1Δ top3Δ sgs1Δ*
dun1Δ sgs1-NΔ	*dun1Δ sgs1-NΔ,HD*

Table 6

Summary of mutant phenotypes

Strain	Growth	with *top3*	with *top1*	with *srs2*	G2 Delay	Meiosis	Hyperrec	with *mec1/ dun1*	HU^r	MMS^r
WT	++++	+	++++	+++	++++	++++	-	++++	++++	++++
*sgs1*Δ	+++	+++	+	-	+++	++	+++	++++	++	++
sgs1-hd	+++	+++	++	-	+++	++	++	++++	+	+
*sgs1-N*Δ	+	-	+	-	+	--	++++	-	++	+
*sgs1-N*Δ*,hd*	+++	+++	+	-	+++	ND	+++	++++	++	+
*top3*Δ	+		- (NQ)	-	+	-	++++	-	-	-

(NQ) = Not quantitated. ND = not determined. Pluses and minuses are relative to wild-type (see Fig. 2 and 3 and Tables 3 to 5 for details). For meiosis, (--) signifies a dominant negative effect.