Bipartite Structure of the *SGS1* DNA Helicase in *Saccharomyces cerevisiae*

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ABSTRACT

SGS1 in yeast encodes a DNA helicase with homology to the human BLM and WRN proteins. This group of proteins is characterized by a highly conserved DNA helicase domain homologous to *Escherichia coli* RecQ and a large N-terminal domain of unknown function. To determine the role of these domains in *SGS1* function, we constructed a series of truncation and helicase-defective (*-hd*) alleles and examined their ability to complement several *sgs1* phenotypes. Certain *SGS1* alleles showed distinct phenotypes: *sgs1-hd* failed to complement the MMS hypersensitivity and hyper-recombination phenotypes, but partially complemented the slow-growth suppression of *top3 sgs1* strains and the *top1 sgs1* growth defect. Unexpectedly, an allele that encodes the amino terminus alone showed essentially complete complementation of the hyper-recombination and *top1 sgs1* defects. In contrast, an allele encoding the helicase domain alone was unable to complement any *sgs1* phenotype. Small truncations of the N terminus resulted in hyper-recombination and slow-growth phenotypes in excess of the null allele. These hypermorphic phenotypes could be relieved by deleting more of the N terminus, or in some cases, by a point mutation in the helicase domain. Intragenic complementation experiments demonstrate that both the amino terminus and the DNA helicase are required for full *SGS1* function. We conclude that the amino terminus of Sgs1 has an essential role in *SGS1* function, distinct from that of the DNA helicase, with which it genetically interacts.

THE *SGS1* gene of *Saccharomyces cerevisiae* is a member of the RecQ family of DNA helicases that includes the human genes *BLM* (Ellis *et al.* 1995), *WRN* (Yu *et al.* 1996), and *RECQL* (Puranam and Blackshear 1994), as well as *rqh1*$^+$ from *Schizosaccharomyces pombe* (Murray *et al.* 1997; Stewart *et al.* 1997). With the exception of *RECQL*, these genes are related by their large size (Figure 1) and by the fact that mutations in them are associated with various forms of genomic instability and sensitivity to DNA-damaging agents (German 1963; Hoehn *et al.* 1975; Gebhart *et al.* 1988; Gangloff *et al.* 1994; Watt *et al.* 1996; Murray *et al.* 1997; Stewart *et al.* 1997). Particular attention has been drawn to this class of genes because of their causal role in the human Bloom's (*BLM*) and Werner's (*WRN*) syndromes. As a model system, the study of *SGS1* in yeast promises to shed light on the role of these proteins in human disease.

Mutations in *SGS1* were first identified by their ability to suppress the slow-growth phenotype of *top3* strains such that *top3 sgs1* double mutants grow at nearly the wild-type rate (Gangloff *et al.* 1994). Curiously, the *sgs1* mutation has the opposite effect in a *top1* background; the *top1 sgs1* double mutant grows more slowly than either single mutant (Lu *et al.* 1996). Two-hybrid screens have detected physical interactions between Sgs1 and both Top3 (Gangloff *et al.* 1994) and Top2 (Watt *et al.* 1995). Compared to wild-type cells, *sgs1* strains show a slight reduction in growth rate and a 10-fold increase in chromosome missegregation (Watt *et al.* 1995). *sgs1* mutants also display elevated recombination: a 7-fold increase in the rate of intrachromosomal recombination at the ribosomal DNA (rDNA) locus and a 3- to 12-fold elevation at *MAT* (Gangloff *et al.* 1994; Watt *et al.* 1996). *sgs1* mutations have been shown to increase the number of extrachromosomal rDNA circles in the cell and, as a result, to cause premature aging (Sinclair and Guarente 1997; Sinclair *et al.* 1997).

The RecQ family proteins have a central domain that is homologous to the bacterial DNA helicase (Figure 1) and as predicted, the BLM, WRN, and Sgs1 proteins have all been shown to possess 3′ to 5′ DNA helicase activity similar to that of RecQ (Umezu *et al.* 1990; Lu *et al.* 1996; Gray *et al.* 1997; Karow *et al.* 1997; Bennett *et al.* 1998). In the case of Sgs1, a region between residues 400 and 1268 was sufficient for helicase activity (Bennett *et al.* 1998). The amino terminus of WRN contains an exonuclease domain (Mushegian *et al.* 1997; Huang *et al.* 1998) that is absent in the other homologs, at least by homology (Figure 1). Two additional conserved regions within the family have been identified by sequence comparison. The Ct domain, which has no known function, is found only in the RecQ family of DNA helicases, while the HRD domain is also found in RNaseD homologs and has been suggested to play a role in binding nucleic acids (Morozov *et al.*

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TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Reference or source
K1875	*MAT***a** *ade2-1 ura3-52 his4-260 trp1-H3 leu2-3,112 lys2-ΔBX-CAN1-LYS2 can1 rDNA::ADE2 rDNA::URA3*	Keil and McWilliams (1993)
W303-1a	*MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Thomas and Rothstein (1989)
AMR60	W303-1α *top3-2::HIS3 sgs1-3::TRP1*	Lu *et al.* (1996)
CHY125	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-12,15 trp1-1 leu2-3,112 can1-100*	Chris Hardy
NJY531	CHY125 *sgs1::loxP*	This study
NJY540	K1875 *sgs1::loxP*	This study
NJY561	*MAT*α *ade2-1 ade3::hisG ura3-1 his3-12,15 trp1-1 leu2-3,112 can1-100 sgs1::loxP slx4::loxP [pJM500::SGS1 URA3 ADE3]*	This study
NJY598	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-12,15 trp1-1 leu2-3,112 sgs1::loxP top1::loxP-KAN-loxP*	This study

1997). Functional nuclear localization signals (NLSs) have been identified at the C termini of both WRN and BLM (Kaneko *et al.* 1997; Matsumoto *et al.* 1997).

We had previously created a helicase-defective allele of *SGS1* (*sgs1-hd*) that changed a single amino acid within the ATP-binding domain of Sgs1 (K706A). Although the mutant protein lacked detectable DNA helicase activity, *sgs1-hd* retained noticeable complementing activity in the *top3 sgs1* and *top1 sgs1* backgrounds (Lu *et al.* 1996). To test whether this generalized to other *sgs1* phenotypes and to more accurately define the functional domains of Sgs1, we performed a structure/function analysis and tested complementation of several *sgs1* phenotypes. We find that certain *SGS1* mutations have distinct phenotypes. For example, *sgs1-hd* is null in a methylmethanesulfonate (MMS) hypersensitivity assay and in a synthetic-lethal assay, but retains partial complementing activity in the *top3 sgs1* background and near wild-type activity in the *top1 sgs1* background. In contrast, an allele encoding the helicase domain alone is unable to complement any *sgs1* phenotype. We show by intragenic complementation that Sgs1 has a bipartite structure, consisting of the DNA helicase domain and an amino terminal domain, both of which are essential for full SGS1 activity.

MATERIALS AND METHODS

Strains: The yeast strains used in this study are listed in Table 1. Strain construction, growth, and transformation followed standard methods (Rose *et al.* 1990). A near-complete deletion of the *SGS1* gene was constructed by amplifying the *loxP-KAN-loxP* cassette with the following oligonucleotides (5′-ATG GTGACGAAGCCGTCACATAACTTAAGAAGGGAGCACAA ATGGCGTACGCTGCAGGTCGAC-3′) and (5′-TCACTTTC TTCCTCTGTAGTGACCTCGGTAATTTCTAAAACCTCGAT CGATGAATTCGAGCTCG-3′) as described (Wach *et al.* 1994; Guldener *et al.* 1996). The PCR product was transformed into strains CHY125 and K1875, its proper integration was verified, and the *KAN* marker was excised to create strains NJY531 and NJY540, respectively. The resulting *sgs1::loxP* deletion removes all but 15 codons of *SGS1* at each end of the gene. Strain NJY598 was constructed by disrupting the *TOP1* gene of strain NJY531 using the same approach and the following oligonucleotides (5′-GGGAGGGCAGAGCTCGAAACTT GAAACGCGTAAAAATGACTATTGCAGCTGAAGCTTCGTA CGC-3′) and (5′-TGCGAACTTGATGCGTGAATGTATTTGC TTCTCCCCTATGCTGCGGCATAGGCCACTAGTGGATCT G-3′). The resulting *top1::loxP-KAN-loxP* disruption removes all codons from the *TOP1* gene.

Recombination assay: NJY540 cells carrying the integrated versions of the Sgs1 N- and C-terminal deletion series were grown on −ade −ura −leu plates, resuspended in water, diluted appropriately, plated onto YPD + ADE plates at ~200 cells per plate, and grown at 30°. After 3 days, seven colonies from each strain were picked, resuspended in 150 μl water, diluted, and aliquoted onto four plates: −ura and YPD at 400 cells/plate, synthetic complete medium containing 5-fluoroorotic acid (5-FOA) at 4000 cells/plate, and synthetic complete medium containing canavanine in place of arginine at 40,000 cells/plate. After 3 days at 30°, colonies were counted. The frequency of viable cells (as determined from the YPD plates) that were canavanine^R or 5-FOA^R (ura$^-$) was determined as described previously and the mean value was presented as a percentage (Christman *et al.* 1993; Gangloff *et al.* 1994). The experiment was performed five times and a representative experiment is presented in Table 3.

Plasmid constructions: Plasmid pJL31 (Lu *et al.* 1996) contains the *SGS1* gene on a 4.5-kb *Xho*I/*Sac*I fragment in pRS415 (Sikorski and Hieter 1989). The natural translation start site of *SGS1* in this plasmid was mutated to the context of an *Nde*I site by ligating a 0.15-kb promoter region of *SGS1* (*Xho*I-*Nde*I PCR fragment) to the first 603 codons (*Nde*I/*Hin*dIII PCR fragment) and to *Xho*I/*Hin*dIII cut pJL31 to create pSM100 (Table 2). To generate N-terminal deletion plasmids, *SGS1* fragments were amplified off pJM505 (an *SGS1* clone from a *CEN-LEU2* library) using *Nde*I-containing forward (sense) oligos that were designed to create new ATG start sites after the indicated residues of *SGS1*, together with an appropriate downstream (antisense) oligo containing either a *Hin*dIII or *Sac*I restriction site. PCR products were digested with *Nde*I and either *Hin*dIII or *Sac*I and subcloned into the corresponding sites of pSM100. To generate C-terminal deletions, reverse (antisense) oligos were designed to place a stop codon after the indicated residues, followed by *Bam*HI and *Sac*I sites. These oligos were used with an appropriate upstream (sense) oligo to amplify fragments that were subcloned into pSM100 as described above. Helicase-defective derivatives were made by subcloning the K706A mutation from pJL37 (Lu *et al.* 1996) on *Nde*I/*Eag*I or *Hin*dIII/*Eag*I fragments into the corresponding

TABLE 2

Plasmids used in this study

Name	Insert	Vector	Reference or source
pRS plasmids			Sikorski and Hieter (1989)
pJM500	*SGS1/ADE3*	pRS416	This study
pJM555	*TOP3/ADE3*	pRS416	This study
pJM505	*SGS1*	p366	American Type Culture Collection library
pSM100	*SGS1*	pRS415	This study
pSM106	*sgs1-ΔN50*	pSM100	This study
pSM107	*sgs1-ΔN101*	pSM100	This study
pSM102	*sgs1-ΔN158*	pSM100	This study
pSM103	*sgs1-ΔN322*	pSM100	This study
pSM104	*sgs1-ΔN484*	pSM100	This study
pSM105	*sgs1-ΔN644*	pSM100	This study
pBS1	*sgs1-ΔC795*	pSM100	This study
pBS5	*sgs1-ΔC300*	pSM100	This study
pBS6	*sgs1-ΔC200*	pSM100	This study
pSM100-hd	*sgs1-hd*	pSM100	This study
pSM106-hd	*sgs1-ΔN50-hd*	pSM100	This study
pSM107-hd	*sgs1-ΔN101-hd*	pSM100	This study
pSM102-hd	*sgs1-ΔN158-hd*	pSM100	This study
pSM103-hd	*sgs1-ΔN322-hd*	pSM100	This study
pSM104-hd	*sgs1-ΔN484-hd*	pSM100	This study
pSM100-HA	*SGS1-HA*	pSM100	This study
pSM100-hd-HA	*sgs1-hd-HA*	pSM100	This study
pSM102-HA	*sgs1-ΔN158-HA*	pSM100	This study
pSM103-HA	*sgs1-ΔN322-HA*	pSM100	This study
pSM105-HA	*sgs1-ΔN644-HA*	pSM100	This study
pJM1541	*sgs1-ΔC795-HA*	pSM100	This study
pJM1547	*sgs1-ΔC795-myc*	pRS414	This study
pSM108	*sgs1-hd*	pRS414	This study
pSM109	*sgs1-ΔN158*	pRS414	This study
pSM110	*sgs1-ΔN322*	pRS414	This study
pSM111	*sgs1-ΔN644*	pRS414	This study
pSM112	*sgs1-ΔC795*	pRS414	This study
pSM113	*sgs1-ΔC997*	pRS414	This study
pSM114	*sgs1-ΔC1247*	pRS414	This study
pJM526	*SGS1*	pRS405	This study
pJM511	*sgs1-hd*	pRS405	This study
pJM531	*sgs1-ΔN50*	pRS405	This study
pJM532	*sgs1-ΔN101*	pRS405	This study
pJM527	*sgs1-ΔN158*	pRS405	This study
pJM528	*sgs1-ΔN322*	pRS405	This study
pJM529	*sgs1-ΔN484*	pRS405	This study
pJM530	*sgs1-ΔN644*	pRS405	This study
pRL1	*sgs1-ΔC795*	pRS405	This study
pRL2	*sgs1-ΔC500*	pRS405	This study
pRL3	*sgs1-ΔC451*	pRS405	This study
pRL4	*sgs1-ΔC400*	pRS405	This study
pRL5	*sgs1-ΔC300*	pRS405	This study
pJM512	*sgs1-ΔC200*	pRS405	This study
pJM6702	*GAL 1/10* p	pRS415	This study
pJM6710	*SGS1*	pJM6702	This study
pJM6715	*sgs1-ΔN484*	pJM6702	This study
pJM6716	*sgs1-ΔN644*	pJM6702	This study
pJM6735	*sgs1-ΔN1000*	pJM6702	This study
pJM6734	*sgs1-ΔC1247*	pJM6702	This study
pJM6727	*sgs1-ΔC300*	pJM6702	This study
pJM6731	*sgs1-ΔC200*	pJM6702	This study
pJM6717	*sgs1-hd*	pJM6702	This study
pJM6722	*sgs1-ΔN484-hd*	pJM6702	This study
pJM6730	*sgs1-ΔC300-hd*	pJM6702	This study
pJM6732	*sgs1-ΔC200-hd*	pJM6702	This study

sites of the recipient plasmid. The inserts of pSM100 derivatives were moved on *Apa*I/*Sac*I fragments (with promoter) into the respective sites of pRS405 and pRS414. Plasmid pJM6702 was constructed by amplifying the *GAL1-10* promoter of pBM272 (Johnston and Davis 1984) with oligonucleotides that introduce *Nde*I, *Bam*HI, and *Sac*I cloning sites adjacent to the *GAL10* promoter and ligating this modified promoter to pRS415. Truncated *SGS1* inserts were moved from pSM100 derivatives into this vector on *Nde*I/*Bam*HI or *Nde*I/*Sac*I fragments.

A C-terminal triple-HA epitope was added to the *SGS1* gene of pSM100 by mutating the stop codon to a unique *Not*I restriction site (resulting in the addition of residues GGR to the C terminus of Sgs1) and ligating a 117-bp *Not*I/*Sac*I fragment encoding 34 additional residues to Sgs1. Subsequent N-terminal deletion derivatives were constructed by moving truncation alleles on *Nde*I/*Sac*II cassettes. The *sgs1-ΔC795-HA* and *sgs1-ΔC795-myc* alleles were made by fusing *Not*I/*Sac*I epitope cassettes to *sgs1-ΔC795* in which the sequence following residue 652 was similarly mutated to a *Not*I restriction site. The 13x-myc epitope was amplified as a *Not*I/*Sac*I fragment using plasmid pFA6a-13Myc-kanMX6 (Longtine *et al.* 1998) as template. Plasmid pJM555 was constructed by ligating the *ADE3* (4.5-kb *Bam*HI/*Sal*I fragment) and *TOP3* (2.5-kb *Sal*I/*Sac*I fragment) genes into the *Bam*HI and *Sac*I sites of pRS416. All PCR reactions were carried out with Vent DNA polymerase and 12 cycles of amplification and used directly without sequencing. The absence of PCR-generated mutations was confirmed in several cases by intragenic complementation. In the case of *sgs1-hd*, the K706A mutation was reverted by site-directed mutagenesis and found to have wild-type activity.

***MMS* sensitivity:** Methylmethanesulfonate was added to a final concentration of 0.012% in YPD agar before pouring and the plates were used within 3 days. Cells were scraped from freshly growing plates, resuspended in water, and OD_{600} was determined. Cells were transferred to microtiter plates in a vertical row at OD = 3.0 and serially diluted 1:5. A replica plater was then used to transfer a drop from each well to MMS and YPD plates.

Extract preparation and immunoblotting: Yeast cells were grown in 50 ml selective media to an OD_{600} = 1, harvested, washed with an equal volume of water, and resuspended in 1 ml A buffer [25 mm Tris (pH 7.5), 1 mm EDTA, 0.01% NP-40, 10% glycerol, 0.1 mm PMSF, and 1 mm DTT] with 0.1 m NaCl and the following protease inhibitors: pepstatin, 10 μg/ml; leupeptin, 5 μg/ml; benzamidine, 10 mm; bacitracin, 100 μg/ml; aprotinin, 20 μg/ml; and sodium metabisulfite, 10 mm. Cells were lysed by vortexing with glass beads at 4° and the extract was collected following centrifugation for 15 min at 4°. Extract concentrations were normalized using the Bradford assay (Bio-Rad, Richmond, CA). One hundred microliters of extract was diluted with an equal volume of RIPA buffer (Harlow and Lane 1988) and incubated with 1 μl anti-HA antibody (5 mg/ml; Boehringer Mannheim, Indianapolis) for 1 hr at 4° prior to incubation with protein A beads (Pharmacia, Piscataway, NJ) for 1 hr at 4°. Immunoprecipitations with anti-myc antibody were done under identical conditions except that 1 μl anti-myc antibody (5 mg/ml; Boehringer Mannheim) was used and protein G-agarose (Pharmacia) was used as precipitant. The beads were washed with RIPA buffer (1 ml, three times), boiled in sample buffer, and the immunoprecipitated material was resolved by 12.5% PAGE. Gels were transferred as described (Towbin *et al.* 1979) and immunoblotted with anti-HA or anti-myc antibody (1:10,000), HRP conjugated secondary antibody (1:10,000; GIBCO BRL, Gaithersburg, MD), and treated with chemiluminescent developer as described by the manufacturer (Amersham, Arlington Heights, IL).

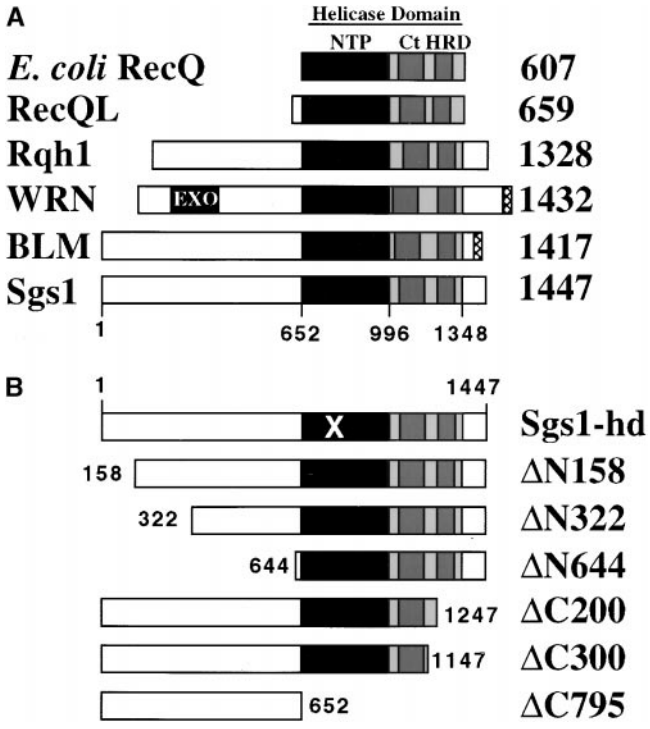

Figure 1.—RecQ family of DNA helicases. (A) Schematic alignment. Protein sizes are shown at right in amino acids. The DNA helicase domain is composed of an NTP-binding motif (black), which shows the greatest similarity between the eukaryotic homologs (39–47% identity) while the 350 amino acids C-terminal to the NTP-binding motif (gray) are less well conserved (22–28% identity). The Ct and HRD domains are shown as dark gray boxes. Open boxes represent domains unique to the eukaryotic homologs. An exonuclease domain (EXO) is present in the WRN protein and nuclear localization sequences are located at the C termini of WRN and BLM (cross-hatched). (B) Sgs1 derivatives used in this study. Sgs1 truncations are named according to the number of amino acids deleted from the wild-type protein. Sgs1-hd contains the K706A mutation.

RESULTS

To identify functional domains in Sgs1, a near-complete deletion of the *SGS1* gene was constructed in strain K1875 to create NJY540 (*sgs1::loxP*; Table 1 and materials and methods). A series of truncation derivatives of the *SGS1* gene was also constructed and several representative alleles are diagrammed in Figure 1B. To express these truncations, a plasmid containing the *SGS1* open reading frame and 150 bp of its promoter was prepared in which the initiating methionine codon was mutated to a unique *Nde*I restriction site (pSM100). This parent vector then served as recipient for *SGS1* truncations on *Nde*I/*Sac*I cassettes that were generated by PCR amplification.

Domains of *SGS1* required for growth in the presence of MMS: *sgs1* mutants have been reported to be sensitive to DNA-damaging agents (Sinclair and Guarente 1997). We confirmed that strain NJY540 (*sgs1::loxP*) was hypersensitive to MMS and tested whether mutant *SGS1*

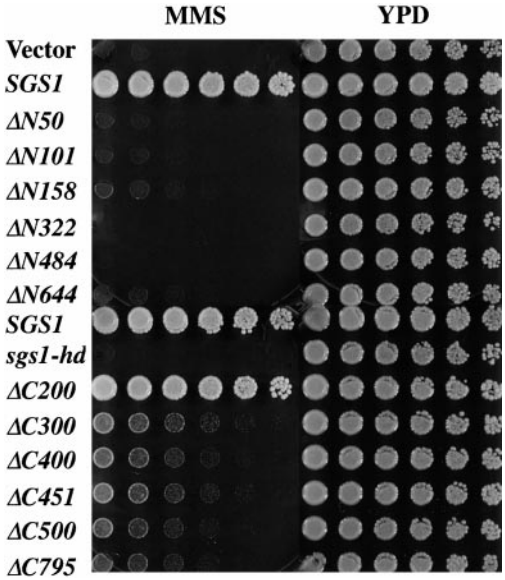

Figure 2.—Complementation of the *sgs1* MMS-hypersensitive phenotype by *SGS1* truncation alleles. Strain NJY540 (*sgs1::loxP*) was stably transformed with the indicated *SGS1* truncation alleles in the *LEU2* integrating vector pRS405. Transformants were resuspended at equal concentrations and spotted in fivefold serial dilutions on YPD plates with and without MMS. Cells were grown and photographed following 2 days at 30°.

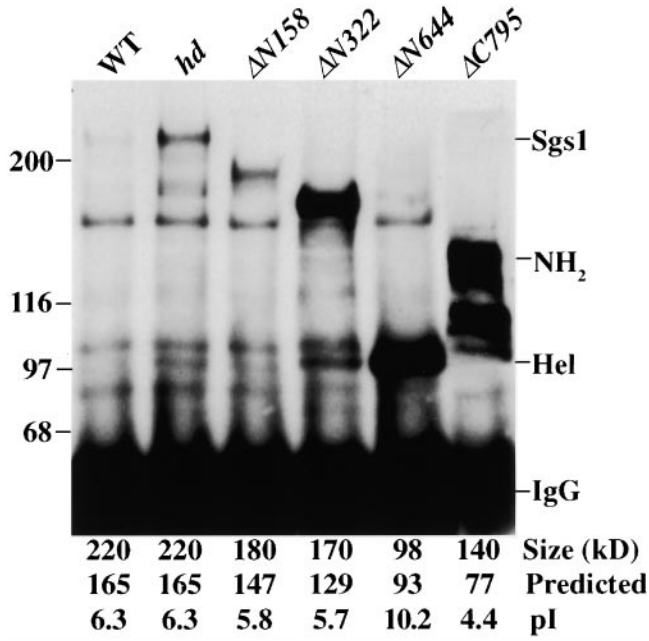

Figure 3.—Expression of Sgs1 truncation derivatives. Strain NJY531 (*sgs1::loxP*) was transformed with the indicated HA-tagged Sgs1 derivatives (top) in the *LEU2/CEN/ARS* vector pRS415. Crude protein extracts were immunoprecipitated and immunoblotted with anti-HA antibody. The positions of full-length Sgs1, the amino-terminal domain (NH_2), and the helicase domain (Hel) are shown along with the IgG heavy chain. The relevant physical data of the expressed proteins are shown at the bottom.

alleles could complement this phenotype. *SGS1* truncation alleles were made in pRS405, integrated at the *LEU2* locus of NJY540, and serial dilutions of the transformants were spotted onto plates containing MMS. As expected, vector alone did not allow growth on this medium, whereas wild-type *SGS1* allowed good growth (Figure 2). None of the amino-terminal truncation alleles conferred resistance to MMS, indicating an essential role of the N terminus. The *sgs1-hd* allele was null in this assay, showing that helicase activity is also required for MMS resistance. A C-terminal truncation of 200 amino acids (Δ*C200*) allowed wild-type growth, although proteins with larger C-terminal deletions were noticeably defective. These larger C-terminal truncation alleles, such as Δ*C795*, allowed limited growth on MMS, but not wild-type levels. We conclude that full MMS resistance requires DNA helicase activity and all but the last 200 amino acids of Sgs1. We also note that whereas *sgs1-hd* is null, the allele Δ*C795*, which completely lacks the helicase domain, retains partial activity. This suggests that the *sgs1-hd* mutation may alter the function of the N terminus or the stability of the protein.

To address whether these mutations affected Sgs1 expression levels, a C-terminal triple-HA epitope tag was added to several truncated Sgs1 proteins and their relative expression levels were compared by immunoblot. Signals obtained by immunoblotting crude extracts were very weak, so consecutive immunoprecipitations and immunoblots were used to enhance these signals. We observed that wild-type Sgs1-HA protein migrated at 220 kD by SDS-PAGE (Figure 3). This apparent molecular weight is larger than the predicted size of 165 kD and may be due to the highly acidic amino terminus. All of the mutant proteins were stable and in some cases were overexpressed relative to the wild type; sgs1-hd and ΔN158 protein levels were slightly elevated, while ΔN322, ΔN644, and ΔC795 levels were greatly elevated. The ΔN158 and ΔN322 proteins also migrated more slowly than expected, while the ΔN644 protein, which consists of the very basic helicase domain alone, migrated at its expected size. The ΔC795 protein, which consists of the very acidic amino-terminus alone, migrated much slower than expected and was associated with a variable amount of cleaved product migrating at 110 kD. We conclude that the mutant alleles are well expressed and any loss of activity we observe is likely due to the loss of functional domains. This idea is further supported by intragenic complementation experiments described below. We also note that any potential complementing activity by these alleles might depend on their elevated expression.

Complementation of the *sgs1 slx4* synthetic lethality: We have identified several nonessential *SLX* genes that, when mutant, cause the cell to require *SGS1* for viability (J. R. Mullen, V. Kaliraman and S. J. Brill, unpublished results). A strain that is null in both *SGS1* and

TABLE 3

Complementation of the *sgs1* hyper-recombination phenotype

Complementing DNA	Percentage marker loss	
	lys2::CAN1::LYS2	*RDN1::URA3::RDN1*
SGS1	0.03 ± 0.01 (1.0×)	1.23 ± 1.78 (1.0×)
Vector	0.16 ± 0.03 (5.3×)	11.6 ± 6.5 (9.4×)
sgs1-hd	0.15 ± 0.04 (5.0×)	11.5 ± 18.0 (9.3×)
sgs1-ΔN158	0.49 ± 0.25 (16×)	14.0 ± 7.6 (11.4×)
sgs1-ΔN158-hd	0.27 ± 0.15 (9.0×)	ND
sgs1-ΔN322	0.17 ± 0.07 (5.6×)	12.6 ± 7.7 (10.2×)
sgs1-ΔN644	0.16 ± 0.04 (5.3×)	13.6 ± 4.6 (11.1×)
sgs1-ΔC200	0.04 ± 0.01 (1.3×)	1.14 ± 1.34 (0.9×)
sgs1-ΔC300	0.16 ± 0.04 (5.3×)	10.0 ± 2.3 (8.1×)
sgs1-ΔC795	0.05 ± 0.01 (1.7×)	2.35 ± 1.40 (1.9×)

Strain NJY540 (*sgs1::loxP*) containing the *URA3* and *CAN1* genes integrated at *RDN1* and *LYS2*, respectively (Keil and McWilliams 1993), was stably transformed with the indicated *SGS1* alleles. After 3 days growth in the absence of selection, the percentage of cells that were canavanine^R or ura$^-$ was determined. The data are presented as the mean of seven independent colonies with the standard deviation and fold increase over wild type. ND, not determined.

SLX4 (YLR135W) is inviable but can be maintained by a copy of *SGS1* on a *URA3*-based plasmid. The strain NJY561 (*slx4 sgs1 pJM500::SGS1 URA3*) is unable to grow on media containing 5-FOA since selection against the *URA3* plasmid is lethal in this background. We tested whether *SGS1* truncations could complement this phenotype by transforming them into NJY561 and streaking the transformants onto media containing 5-FOA. Apart from wild-type *SGS1*, only Δ*C200* was able to complement the loss of *SGS1* in the *slx4::loxP* background (summarized in Table 5). Identical results were obtained with four other *SLX* mutants (data not shown). As in the MMS assay, *SGS1* function requires the N terminus, the DNA helicase domain, and most of the C terminus; only the last 200 amino acids of the protein is dispensible. Finally, this assay appears more stringent than complementation of MMS hypersensitivity since no intermediate complementation was observed with *SGS1* C-terminal truncations larger than Δ*C200*.

Complementation of the *sgs1* hyper-recombination phenotype: Mutations in *SGS1* cause a hyper-recombination phenotype (Gangloff *et al.* 1994; Watt *et al.* 1996). To determine which domains of *SGS1* are required to complement this phenotype, we used strain NJY540 (K1875 *sgs1::loxP*), which contains markers for the measurement of genetic exchange; *CAN1* is inserted at *LYS2*, creating a *LYS2-CAN1-lys2* duplication, and *URA3* is inserted within the rDNA cluster (Keil and McWilliams 1993). *SGS1* truncation alleles were stably integrated in this strain and the frequencies of *URA3* and *CAN1* marker loss were determined after growth on nonselective media (Table 3). Compared to vector alone, transformation with wild-type *SGS1* reduced the frequency of marker loss five- to ninefold. This reduction in marker loss agrees well with the ability of *SGS1* to suppress recombination in other systems (Gangloff *et al.* 1994; Watt *et al.* 1996). As in the first two assays, the *sgs1-hd* allele showed negligible activity.

Interestingly, a truncation of the Sgs1 amino terminus (Δ*N158*) increased the recombination to a level greater than the null frequency (11–16 times over wild type). Over the course of several trials, the enhancement relative to null ranged from 1.2 to 3.3 (2.2 ± 0.9 average) for *URA3* and 0.9 to 3.0 (1.7 ± 1.0 average) for *CAN1*. To determine if this hypermorphic phenotype was dependent on the helicase activity of the Δ*N158* allele, we constructed the compound allele Δ*N158-hd*, which includes the K706A mutation known to inactivate the Sgs1 DNA helicase (Lu *et al.* 1996). Eliminating the helicase activity reduced *CAN1* recombination to nine times the wild-type frequency, which was still 1.7 times the null frequency. Larger N-terminal truncations, such as Δ*N322*, produced recombination frequencies that were equivalent to the null. The smallest C-terminal truncation (Δ*C200*) retained wild-type activity, while a truncation of 300 amino acids (aa; Δ*C300*) was null. Unexpectedly, the Δ*C795* allele, which lacks the entire DNA helicase domain, retained significant complementing activity as it did in the MMS hypersensitivity assay. Taken together, we conclude that (1) the amino terminus of Sgs1 is necessary and sufficient for suppressing recombination; (2) Δ*N158* is a hypermorphic allele, with some of its activity independent of DNA helicase activity; and (3) *SGS1* affects recombination at *RDN1* and *LYS2* similarly. As in the MMS assay, the fact that *sgs1-hd* fails to complement as well as Δ*C795* does suggests that the *-hd* mutation inhibits the activity of the amino terminus.

Complementation in the *top3 sgs1* mutant background: *Role of SGS1 helicase activity:* Given the antagonistic nature of helicase-topoisomerase interactions, it is reasonable to expect that the slow growth of *top3* cells might be caused by the DNA-unwinding activity of Sgs1, unchecked by Top3. However, we previously judged *sgs1-hd* to be active in inhibiting the growth of *top3 sgs1* cells when streaked onto agar plates (Lu *et al.* 1996). To avoid bias in selecting colonies and suppressors that might arise during streak purification, we observed the growth of colonies immediately following transformation of *SGS1* alleles in this background. When a *top3 sgs1* double mutant was transformed with vector alone it formed large colonies that were heterogeneous in size. When transformed with *SGS1* it formed smaller colonies characteristic of the *top3* slow-growth phenotype (Figure 4A). When the double mutant was transformed with *sgs1-hd*, both the number and size of the transformant colonies appeared to be suppressed relative to vector alone. However, these colonies were significantly larger and more heterogeneous than wild-

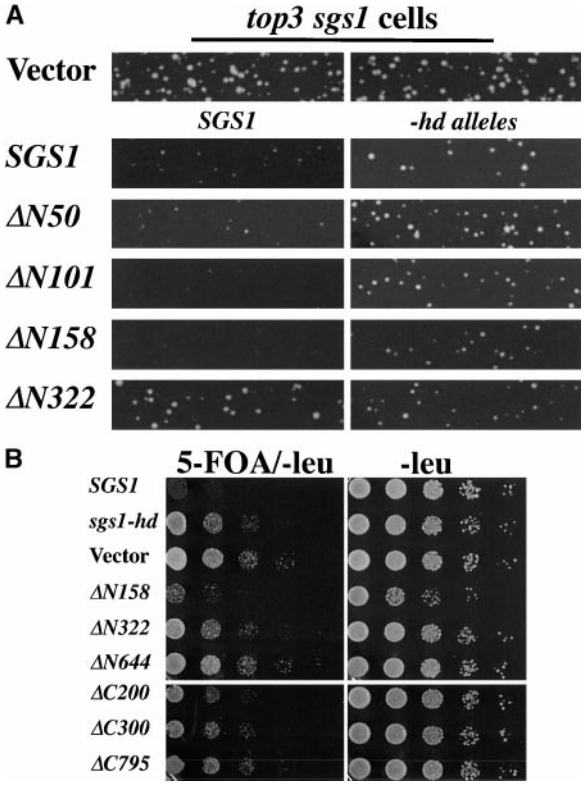

Figure 4.—Complementation of *top3 sgs1* slow-growth suppression by *SGS1* truncation alleles. (A) Strain AMR60 (*top3 sgs1*) was transformed with the indicated *SGS1* truncation alleles (left), or the indicated alleles also containing the K706A helicase-defective mutation (right), in pRS415. Selective plates were photographed following 2 days growth at 30°. (B) Strain AMR60 (*top3 sgs1*) was transformed with pJM555 (*TOP3*/*URA3*) and the indicated *SGS1* alleles in pRS415 (*LEU2*). Transformants were streak purified on SD-leu plates, resuspended at equal concentrations, and spotted in 10-fold serial dilutions on SD-leu plates with or without 1 mg/ml 5-FOA. Cells were grown and photographed following 2 (-leu) or 3 (5-FOA/-leu) days at 30°. The toxic effect of Δ*N158* is apparent in both the absence or presence of 5-FOA.

type *SGS1* colonies, indicating that *sgs1-hd* is not wild type but retains only partial activity in this assay (Figure 4A, top four panels).

To quantitate the activity of *sgs1-hd* in this assay, we used three methods. First, we picked large and small colonies from the transformation plates and measured growth rates in selective liquid medium at 30°. When *top3 sgs1* cells were transformed with vector alone, the large and small colonies grew with roughly similar doubling times of 135 and 140 min, respectively. Cells transformed with *SGS1* also grew at rates reflecting their colony size (205 and 235 min) as did cells transformed with *sgs1-hd* (210 and 155 min). We conclude that the average growth rate of *sgs1-hd* transformants (183 min) is intermediate to that of vector- (138 min) and SGS1- (220 min) transformed cells. In particular, even fast-growing *sgs1-hd* transformants grew slower than vector transformants, indicating that *sgs1-hd* retains partial activity in this assay. As a second method we tested how a population of *top3* cells, not just a few colonies, responded to *sgs1-hd*. To do this, we complemented a *top3 sgs1* double mutant with a *TOP3*/*URA3* plasmid (pJM555) and one of the *SGS1* alleles carried on a *LEU2* plasmid. Strains carrying the two plasmids were then serially diluted and spotted on plates containing 5-FOA, to select against the *TOP3*/*URA3* plasmid, and on control plates. As expected, cells carrying p*SGS1*/*LEU2* grew poorly on 5-FOA/-leucine, displaying the *top3* slow-growth phenotype. Cells transformed with the empty *LEU2* vector grew well on 5-FOA/-leucine, displaying the *top3 sgs1* phenotype of fast growth (Figure 4B). Cells carrying *sgs1-hd* display an intermediate phenotype, requiring an ~10-fold higher concentration of cells to equal the growth obtained with vector alone.

A third method to test the activity of *sgs1-hd* in the *top3 sgs1* background was to place the *SGS1* alleles behind the inducible *GAL10* promoter and observe the effect of overexpression in the double mutant. Transformants were serially diluted and spotted onto selective plates containing either galactose or glucose. Cells transformed with vector alone grew well on both galactose and glucose, while cells transformed with *SGS1* grew slowly on galactose, as expected (Figure 5). If the slow growth of *top3* cells is due solely to Sgs1 helicase activity unbalanced by Top3 activity, then overexpressing *sgs1-hd* should have no effect. However, overexpression of *sgs1-hd* reduced the growth of *top3 sgs1* cells such that 5- to 25-fold more *sgs1-hd* cells were required to equal the overall amount of growth of cells transformed with vector alone (Figure 5, bottom panels). This effect is clearly not as severe as overexpressing wild-type *SGS1*. Furthermore, the intermediate activity of *sgs1-hd* is unlikely to be due to residual helicase activity as Δ*C795*, which encodes only the N terminus, shows the same intermediate activity in this assay (Figure 4B). Presumably, the amino-terminal domain of Sgs1 must also contribute to growth suppression in the absence of Top3.

Role of the SGS1 amino terminus: When the *top3 sgs1* double mutant was transformed with the amino-terminal truncation alleles Δ*N50*, Δ*N101*, and Δ*N158*, we were surprised to find that the transformants grew more slowly than cells transformed with *SGS1* (Figure 4A). In the case of Δ*N158*, growth was so slow that transformant colonies were not visible until 3 days at 30°, compared to *SGS1* transformants that were clearly visible after 2 days. This unusual phenotype of Δ*N158* is discussed further below. Larger truncations, such as Δ*N322* reversed this slow-growth trend while Δ*N644* conferred a null phenotype (Figure 4B). The null phenotype of Δ*N644* again confirms that complementation of *top3 sgs1* slow-growth suppression requires the N terminus of Sgs1 and not just DNA helicase activity. We tested whether overexpression of the helicase domain would overcome this limitation. Overexpression of the N-terminal deletions, up to and including Δ*N644*, did inhibit

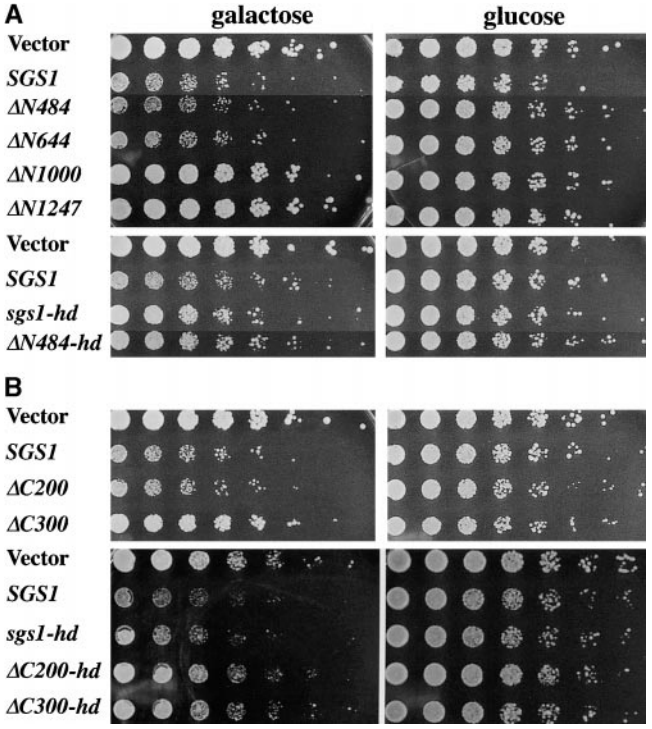

Figure 5.—Overexpression of *SGS1* truncations reveals *sgs1-hd* activity and a role for the C terminus of Sgs1. (A) Strain AMR60 (*top3 sgs1*) was transformed with a galactose expression vector (pJM6702) containing the indicated *SGS1* N-terminal truncation alleles (top) or the indicated alleles also containing the K706A helicase-defective mutation (bottom). Transformants were streak purified on SD-leu plates, resuspended at equal concentrations, and spotted in fivefold serial dilutions on selective plates containing either galactose or glucose. (B) As described above, but with the *SGS1* C-terminal truncation series. Cells were grown and photographed after 2 (glucose) or 3 (galactose) days at 30°.

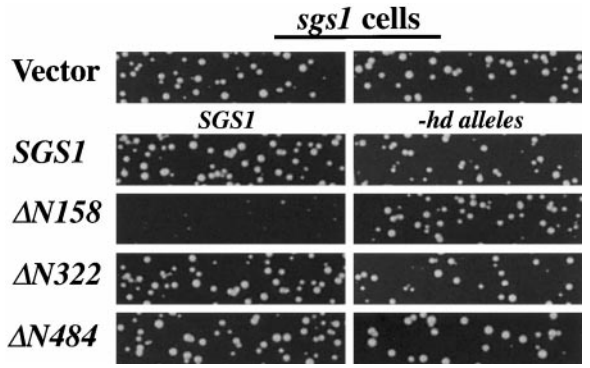

Figure 6.—Truncation of the Sgs1 amino terminus uncovers a helicase-dependent toxicity. (A) Strain NJY531 (*sgs1::loxP*) was transformed with the indicated *SGS1* truncation alleles (left) or the indicated alleles also containing the K706A helicase-defective mutation (right), in pRS415. Cells were grown on SD-leu and photographed following 3 days at 30°.

growth as wild-type *SGS1* did (Figure 5A, top panels and data not shown). Thus, overexpression of the helicase domain alone can suppress the lack of the amino terminus. Accordingly, Δ*N1000*, which removes both the N terminus and much of the DNA helicase domain, is null in this assay. We conclude that complementation of *top3 sgs1* slow-growth suppression requires both the N terminus and DNA helicase activity.

The severe growth inhibition of Δ*N158* described above will be referred to as the "toxic effect" to distinguish it from the normal growth inhibition of wild-type *SGS1* in the *top3* background. The toxic effect of Δ*N158* and its reversal by larger truncations like Δ*N322* correlate with the Δ*N158* hypermorphic phenotype observed in the recombination assay. To test whether this toxicity was specific to the *top3* background, we transformed a *sgs1* single mutant with the truncation series and found that its growth was similarly inhibited by this allele (Figure 6, left panels). We conclude that the toxic effect is specific for *sgs1* mutant cells and that it is recessive since it is not observed in wild-type cells (data not shown). We tested whether the helicase activity of Sgs1 was required for the toxic effect by constructing compound alleles that included the *-hd* mutation. The addition of the *-hd* mutation alleviated the toxic effect of the small amino-terminal truncations in both the *top3 sgs1* (Figure 4A, right-hand panels) and *sgs1* (Figure 6, right panels) backgrounds. In the *top3 sgs1* background, the colony sizes after transformation of the *sgs1-hd*, Δ*N50-hd*, Δ*N101-hd*, and Δ*N158-hd* alleles were similar to each other and intermediate to those obtained with vector and wild-type *SGS1*. These results indicate that the Δ*N158* toxic phenotype requires both the specific amino terminal truncation and DNA helicase activity.

Role of the SGS1 carboxy terminus: Sgs1 C-terminal truncations were tested for complementation in the *top3 sgs1* background. As previously reported, none of the C-terminal truncation alleles, including Δ*C200*, significantly inhibited the growth of the *top3 sgs1* double mutant using a colony size assay (Lu *et al.* 1996; data not shown). To quantitate the defect, we picked large and small colonies from the transformation plates and measured growth rates in selective liquid medium at 30°. Cells transformed with Δ*C200* grew at rates reflecting their colony size (140 and 150 min) with an average doubling time of 145 min, relative to 220 min for *SGS1* and 138 min for vector. Thus, in this assay alone, Δ*C200* does not behave like wild type. Using the galactose overexpression assay, Δ*C200* significantly inhibited growth in *top3 sgs1* cells (Figure 5B, top panels), but this is likely due to excess helicase activity since either larger deletions (Δ*C300*) or inactivation of the helicase domain (Δ*C200-hd*) reduced this effect (Figure 5B, bottom panels). The importance of the C-terminal 200 amino acids is further emphasized by the fact that Δ*C200-hd* fails to inhibit growth as much as *sgs1-hd* when overexpressed. We conclude that complementation of *top3 sgs1* slow-growth suppression is an extremely sensitive assay that requires the N terminus, DNA helicase activity, and the C-terminal 200 amino acids for full *SGS1* activity.

Complementation in the *top1 sgs1* mutant background: *sgs1* and *top1* display a synergistic growth defect

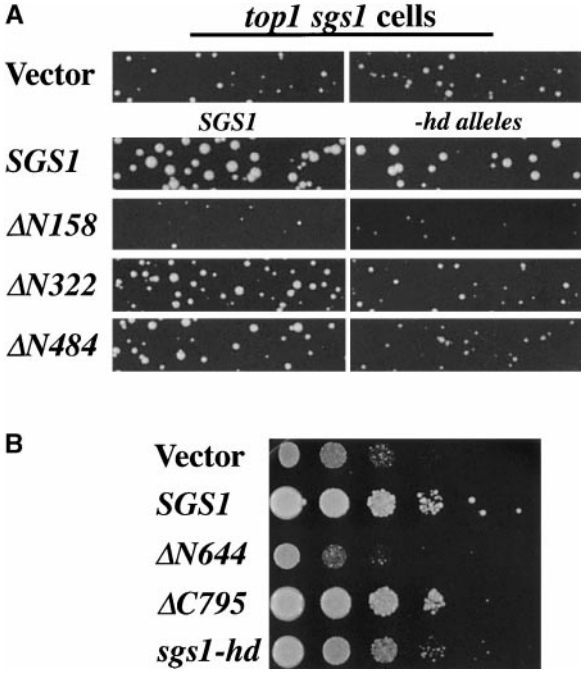

Figure 7.—Complementation of the *top1 sgs1* growth defect does not require the DNA helicase domain of Sgs1. (A) Strain NJY598 (*top1 sgs1*) was transformed with the indicated *SGS1* truncation alleles (left), or the indicated alleles also containing the K706A helicase-defective mutation (right), in pRS415. Cells were grown and photographed following 3 days at 30°. (B) NJY598 transformants containing the indicated *SGS1* alleles in pRS415 were streak purified on SD-leu plates, resuspended at equal concentrations, and spotted in 10-fold serial dilutions on SD-leu plates. Cells were grown and photographed following 4 days at 22°.

such that a *top1 sgs1* double mutant grows more slowly than either single mutant (Lu *et al.* 1996). We transformed *top1 sgs1* cells with our *SGS1* mutant alleles to determine how well they could improve growth in this background. As expected, NJY598 (*top1 sgs1)* formed small colonies when transformed with vector alone and large colonies when transformed with *SGS1* (Figure 7A). Large colonies were also obtained when these cells were transformed with *sgs1-hd*, indicating that helicase activity is not essential in this assay (Figure 7A). We also compared the growth rates of a population of cells by serially diluting transformants and spotting on selective plates. The *top1 sgs1* cells transformed with Δ*C795*, which encodes the N terminus, grew as well as mutants transformed with wild-type *SGS1*, but cells transformed with Δ*N644*, which encodes the helicase, grew at a rate comparable to those transformed with vector alone (Figure 7B). We conclude that DNA helicase activity is completely dispensible in this assay. As in both the MMS hypersensitivity and hyper-recombination assays, complementation by Δ*C795* was better than full-length *sgs1-hd*, suggesting that the *-hd* mutation inhibits the complementing activity of the N terminus.

The toxic effect of Δ*N158* was apparent in the *top1 sgs1* background, since it exacerbated rather than improved the growth defect (Figure 7A). When more of the N terminus was removed, as in the Δ*N322* and Δ*N484* alleles, the toxic effect was lost and complementing activity returned, although not at wild-type levels. Thus, the minimal complementing region is likely to lie between residues 484 and 644 of the N terminus. In contrast to other backgrounds, the toxic effect of Δ*N158* was not alleviated by the Δ*N158-hd* mutation (Figure 7A, right panels). This, however, is consistent with the fact that complementation in this assay does not require helicase activity. Nevertheless, the complementing activity of Δ*N322* and Δ*N484* was adversely affected by the *-hd* mutation (Figure 7A, right panels). We suspect that this result is caused by the inhibitory effect of the *-hd* mutation on the activity of the N terminus. We conclude that, in the *top1 sgs1* background, the toxic effect is independent of helicase activity.

Intragenic complementation by *SGS1*: Because our assays revealed that Sgs1 contained two functional domains that could be independently manipulated, we wanted to determine if the two domains could be physically separated and still function. *SGS1* truncations were subcloned into the appropriate vectors for simultaneous expression and complementation testing of two mutant alleles. Neither Δ*N322* nor *sgs1-hd* individually conferred MMS resistance (Figure 2). However, when these two alleles were expressed simultaneously in *sgs1* cells, they grew on MMS plates at a rate close to wild type (Figure 8, top). We also found that Δ*N322* complemented the C-terminal truncation alleles Δ*C795* and Δ*C997*, while complementation with Δ*C1247* was impaired (Figure 8, top). In the most stringent test, we found that nonoverlapping N- and C-terminal helicase domains could complement. Figure 8 (middle) shows that Δ*N644* (helicase domain) can be complemented by *sgs1-hd* as well as Δ*C795* (N-terminal domain). In fact, significant growth on MMS is obtained with alleles Δ*N644* and Δ*C997*, indicating that residues 450–645 are dispensible for MMS resistance. As described above, Δ*C1247* fails to allow optimal growth in the presence of Δ*N644*. Consistent with these results, Δ*C795* (N-terminal domain) cannot be rescued by *sgs1-hd*, although it can be rescued by other N-terminal truncation alleles, including Δ*N644* (helicase domain; Figure 8, bottom panel).

To confirm that *SGS1* intragenic complementation is not unique to MMS hypersensitivity, we tested the more stringent assay of restoring viability to the synthetic-lethal *slx4 sgs1* strain. Whereas neither Δ*N322* nor *sgs1-hd* could individually restore viability in this background (Table 5), these alleles together rescued the *slx4 sgs1* strain with a growth rate equivalent to wild type (Table 4). Moreover, Δ*N644* (helicase domain) and the Δ*C795* (N-terminal domain) together restored viability to the double mutant, although the growth rate was somewhat reduced. We conclude that Sgs1 can be expressed as functional N-terminal and DNA helicase domains, con-

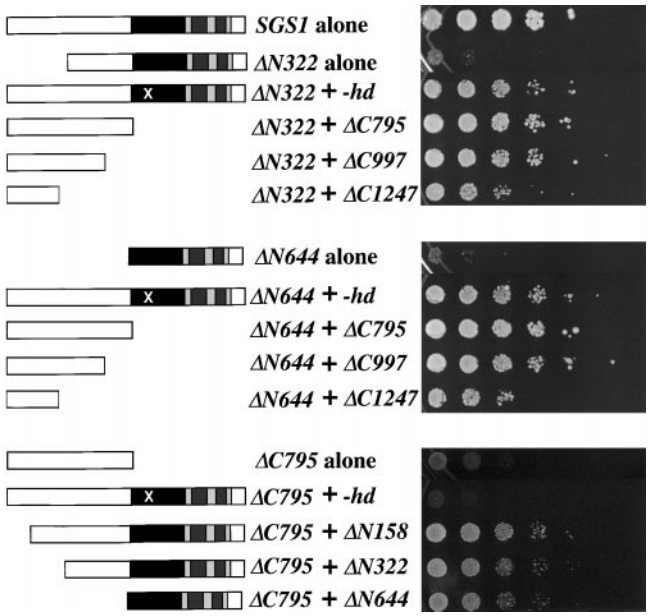

Figure 8.—*SGS1* intragenic complementation. Strain NJY-540 (*sgs1::loxP*) was transformed with one *SGS1* truncation allele in pRS415 (top, pSM103; middle, pSM105; bottom, pBS1) and another *SGS1* truncation allele in pRS414 (top and middle, pRS414, pSM108, pSM112, pSM113, and pSM114; bottom, pSM108, pSM109, pSM110, and pSM111). Transformants were streak purified on plates lacking tryptophane and leucine, resuspended at equal concentrations, and spotted in fivefold serial dilutions on YPD plates with MMS. Plates were photographed following 2 days growth at 30°. Complementation of MMS hypersensitivity by wild-type *SGS1* (pSM100) is shown at the top. On an individual basis, none of the indicated mutant alleles allowed growth on MMS (Figure 2 and data not shown).

sistent with the dual functions of the protein, as revealed by truncation analysis.

Successful intragenic complementation suggests that either the two truncated Sgs1 proteins perform their functions separately or they assemble into a stable complex, as in β-galactosidase α-complementation. To test these possibilities, we placed a myc-epitope tag on the C terminus of the N-terminal domain (Δ*C795-myc*) and expressed it with the Sgs1-HA-tagged proteins described in Figure 3. Crude extracts were made from cells coexpressing these proteins and the potential complexes were analyzed by consecutive immunoprecipitation (IP) and immunoblotting. In this experiment Sgs1-HA migrated as a doublet at 220 kD perhaps due to post-translational modification (Figure 9, top). When full-length Sgs1-HA was coexpressed with ΔC795-myc and immunoprecipitated with anti-myc antibody, a small amount of Sgs1-HA could be detected in the IP following blotting with anti-HA antibody (Figure 9, middle). This result suggests that Sgs1 interacts with itself in the cell, perhaps forming a multimer via the N-terminal domain. Consistent with this idea, the HA-tagged N-terminal truncation alleles failed to coprecipitate with ΔC795-myc. A control immunoblot with anti-myc antibody revealed that ΔC795-myc was expressed in these strains (Figure 9, bottom panel). These results suggest that the N terminus and helicase domain expressed during intragenic complementation do not associate in the cell and that they perform their functions separately. We cannot, however, rule out the possibility that the truncated Sgs1 proteins were complexed in the cell and were disrupted by the lysis or immunoprecipitation procedures.

DISCUSSION

As a first step toward characterizing the RecQ family of DNA helicases, we have conducted a structure/function analysis of the yeast *SGS1* gene. Table 5 summarizes the results of our experiments. An unanticipated finding of these studies was that certain *SGS1* alleles have distinct phenotypes depending on the assay. In assays measuring recombination or growth rates, we identified alleles with intermediate or null phenotypes in addition to hypermorphic alleles whose phenotype was more severe than the null. In contrast, in assays measuring MMS resistance or complementation of synthetic lethality, most of the intermediate and hypermorphic alleles displayed a null phenotype. Further evidence that these alleles retained partial activity was that wild-type function could be restored through intragenic complemen-

TABLE 4

Intragenic complementation of *sgs1 slx4* synthetic lethality

Complementing DNA	Vector	*sgs1-hd*	*sgs1-ΔC795*	*sgs1-ΔC997*	*sgs1-ΔC1247*
SGS1	++	++	++	++	++
Vector	—	—	—	—	—
sgs1-ΔN158	—	++	++	++	—
sgs1-ΔN322	—	++	++	++	—
sgs1-ΔN644	—	+	+	—	—

Strain NJY561 (*sgs1 slx4*) carrying plasmid pJM500 (*SGS1/URA3/ADE3*) was cotransformed with the indicated *SGS1* alleles on *LEU2* (rows) and *TRP1* (columns) plasmids as in Figure 8. The transformants were then streaked onto synthetic complete medium containing 1 mg/ml 5-FOA. Wild-type growth on this medium is indicated by ++.

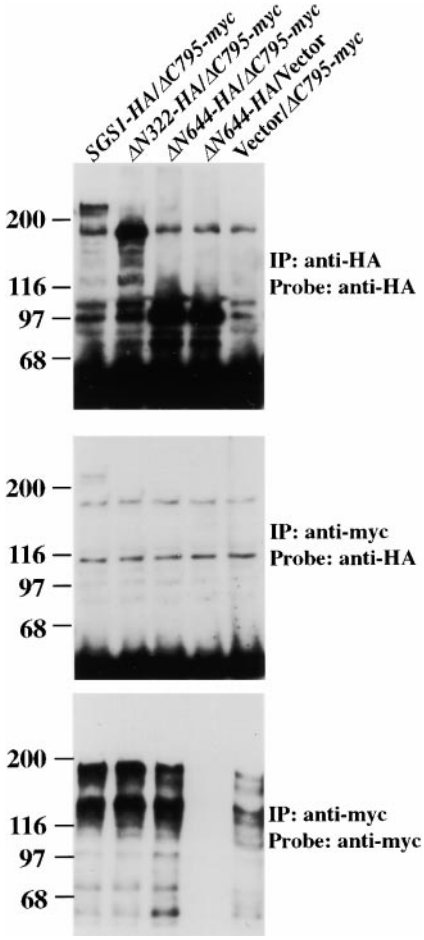

Figure 9.—Sgs1 domains fail to interact physically. Strain NJY531 (*sgs1::loxP*) was transformed with the indicated *SGS1-HA* truncation alleles and Δ*C795-myc* on pRS415 and pRS414, respectively. Cells were harvested and protein extracts were prepared for immunoprecipitation and immunoblotting with the indicated antibodies.

tation. We believe that certain mutant alleles display distinct phenotypes for two reasons: first, some assays differ in their sensitivity to Sgs1 activity and second, some assays require only one of Sgs1's two functional domains.

Compared to the null allele, Δ*N158* exhibited both very high rates of recombination and toxicity in *sgs1* strains, yet was null in the MMS and synthetic lethality assays (Table 5). The toxicity was most apparent in *top1 sgs1* cells, where we expected any *SGS1* activity to improve, not inhibit growth. And while *SGS1* activity is expected to inhibit growth in the *top3* background, Δ*N158* inhibited growth even more dramatically than wild-type *SGS1*. The mechanism of Δ*N158* toxicity and hyper-recombination is not understood, but it is known that overexpressing wild-type *SGS1* leads to severe growth inhibition in these backgrounds (Figure 5; data not shown). Therefore, a possible explanation for the Δ*N158* phenotype is that truncation of the N terminus creates a "hyperactive" protein. This idea is consistent with the simultaneous reduction in recombination rate and toxicity caused by the larger truncation encoded by Δ*N322* or by a mutation in the helicase domain.

To explain these data we propose that an inhibitory domain of Sgs1 is removed by Δ*N158*, revealing a stimulatory domain that, in turn, is removed by Δ*N322* (Figure 10A). Since removal of the stimulatory domain correlates with increased ΔN322 protein levels (Figure 3), the loss of the hypermorphic phenotype must be due to loss of function and not a loss of protein stability. A prediction of this model is that overexpression of wild-type *SGS1* should stimulate recombination and other *sgs1* phenotypes. Indeed, overexpression of *SGS1* is known to induce nucleolar fragmentation, a phenotype of prematurely aging *sgs1* cells (Sinclair *et al.* 1997). Does the Δ*N158* hypermorphic phenotype depend on DNA helicase activity? The toxicity of Δ*N158* in *sgs1* and *top3 sgs1* cells is relieved by a point mutation in the helicase domain. However, Δ*N158-hd* remains toxic in *top1 sgs1* cells and causes excessive hyper-recombination in *sgs1* cells even in the absence of helicase activity. Thus, the hypermorphic phenotype depends on helicase activity only when the specific assay depends on helicase activity.

How might the domain between residues 1 and 158 function to inhibit the activity of Sgs1? The simplest model (Figure 10B, top) proposes that a subdomain of the N terminus, N1, inhibits the DNA helicase (pathway 1), while the alternative, which we prefer, is that N1 inhibits an activity present in N2 (pathway 2). An obvious possibility is that N2 is a nuclease as in WRN (Huang *et al.* 1998), although other activities are conceivable. A second model (Figure 10B, bottom) proposes that Sgs1 exists in a complex of proteins and that N1 and N2 interact with hypothetical repressors or targeting proteins, respectively.

The Δ*C795* allele, which expresses the N-terminal 652 aa alone, restored good growth to a *top1 sgs1* double mutant, consistent with our previous report that a full-length helicase-defective allele was active in this assay (Lu *et al.* 1996). The complementing region of the amino terminus may lie C-terminal to the first 484 amino acids, since Δ*N484* was also able to restore good growth to *top1 sgs1* cells. We conclude that the residues between 484 and 652 contain a domain that is important for good growth in the *top1* background. How could this domain function to overcome a Top1 deficiency? One possibility is that it recruits an alternate topoisomerase, such as Top2, to relax excess superhelicity. Such an idea is consistent with the fact that residues 434–744 of Sgs1 were found to interact with Top2 in a two-hybrid screen (Watt *et al.* 1995).

When expressed under its own promoter, Δ*C795*, encoding the amino terminus alone, showed at least partial activity in complementing four phenotypes: MMS hypersensitivity, hyper-recombination, *top3 sgs1* growth suppression, and *top1 sgs1* slow growth. In contrast, Δ*N644*,

TABLE 5

Complementation by SGS1 truncation alleles

Allele	MMS hypersensitivity	*slx4* lethality[a]	Hyper-recombination	*top3 sgs1* slow-growth suppression: Liquid growth	*top3 sgs1* slow-growth suppression: FOA/-leu growth	*top3 sgs1* slow-growth suppression: Gal over-expression	*top1 sgs1* slow growth
sgs1::loxP	−	−	−	−	−	−	−
SGS1	+	+	+	+	+	+	+
sgs1-hd	−	−	−	+/−	+/−	+/−	+/−
Δ*N158*	−	−	H[b]	ND	H	ND	H
Δ*N322*	−	−	−	ND	+/−	ND	+
Δ*N484*	−	−	ND	ND	ND	+	+
Δ*N644*	−	−	−	ND	−	+	−
Δ*C200*	+	+	+	+/−	+/−	+	+
Δ*C300*	+/−	−	−	ND	−	−	−
Δ*C795*	+/−	−	+/−	ND	+/−	ND	+

[a] Strain NJY561 (*sgs1 slx4*) carrying plasmid pJM500 (*SGS1/URA3/ADE3*) was transformed with the indicated *SGS1* alleles in pRS415. The transformants were then streaked onto synthetic complete medium containing 1 mg/ml 5-FOA.
[b] Hypermorphic phenotype.
+, full complementation; −, no complementation; +/−, intermediate complementation; ND, not determined.

which encodes the helicase domain alone, was null in these assays. Since intragenic complementation experiments demonstrated that the Δ*N644* allele was expressed, we must conclude that the N terminus is at least as important to Sgs1 function as is the DNA helicase. This is surprising given that *SGS1* is epistatic to *TOP3* and it is thought that Sgs1 unwinds DNA strands to create a substrate for Top3 (Gangloff *et al.* 1994). Indeed, in the *Escherichia coli* system it has been shown that RecQ generates a substrate for strand passage by DNA topoisomerase III (Harmon *et al.* 1999). A possible explanation for this discrepancy is that full-length Sgs1 may be required *in vivo* to maintain the integrity of a larger protein complex (*e.g.*, Sgs1, Top1, and Top2). Alternatively, Sgs1 may modify DNA as well as unwind it for processing by eukaryotic Top3. We suggest that the N terminus of Sgs1 has its own activity, such as a nuclease or perhaps a unique DNA-binding activity, which is an important component of *SGS1* function.

The C-terminal truncation series revealed that the last 200 aa of Sgs1, which include the HRD domain, are dispensible for all phenotypes except complementation of *top3 sgs1* slow-growth suppression. However, overexpression of Δ*C200* causes slow growth in this background, as does Δ*N644* (Table 5), indicating that an imbalance of helicase over Top3 activity can induce slow growth even in the absence of the N terminus. This is consistent with the fact that *sgs1-hd* lacks full complementation in this assay and shows that helicase activity is an important, although not exclusive, determinant in this assay. The failure of Δ*C200* to complement would be expected if the C-terminal 200 aa are important for protein localization as in WRN (Matsumoto *et al.* 1997). Since localization did not appear to be a problem in other assays, we suspect that the C-terminal domain has another activity. One possibility is that HRD stimulates the activity of *SGS1* by binding to DNA or RNA as proposed originally (Morozov *et al.* 1997), and that the

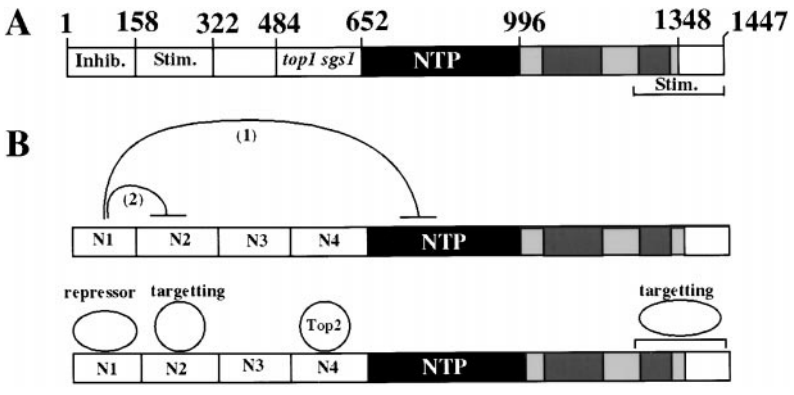

Figure 10.—*SGS1* structure/function models. (A) The Sgs1 protein is presented schematically with functional domains shown in the N and C termini. The domain between residues 484 and 652, labeled "*top1 sgs1*," represents the region required for good growth in the *top1 sgs1* double mutant. (B) Models of *SGS1* regulation. The upper model proposes a direct mechanism for SGS1 regulation in which the inhibitory domain, N1, inhibits the DNA helicase domain (1) or the putative stimulatory region, N2 (2). The lower model proposes an indirect mechanism for Sgs1 regulation in which activity is controlled by other factors. In this case, N1 interacts with a specific repressor protein while the stimulatory domains, N2 and the C terminus, interact with proteins involved in targeting Sgs1 to a protein complex or DNA substrate. The N4 domain is proposed to mediate interaction with Top2 (Watt *et al.* 1995).

requirement for this activity is only observed in the very sensitive *top3 sgs1* slow-growth suppression assay.

The *sgs1-hd* allele, which carries a point mutation in the helicase domain, was null in three assays and intermediate in complementing the *top1 sgs1* growth defect and *top3 sgs1* slow-growth suppression. In some of these assays (*e.g.*, MMS hypersensitivity, hyper-recombination, and complementation of *top1 sgs1* slow growth), *sgs1-hd* was more defective than Δ*C795*, which lacks the entire helicase domain. We suspect that the defective helicase domain inhibits the function of the amino terminus. This could be explained simply if the helicase domain interacts with other proteins such as Top3 (Gangloff *et al.* 1994) or Top2 (Watt *et al.* 1995). The interaction of *sgs1-hd* with these proteins might tether or restrict the amino terminus from performing its normal function. Alternatively, the presence of an inactive helicase in this complex may inhibit these other enzyme activities, contributing to the *sgs1-hd* phenotype.

The dual functions of Sgs1 may extend to other RecQ family members such as the *rqh1*$^+$ gene of *S. pombe*. The *rqh1.r12* allele contains a single missense mutation in the ATP-binding domain of Rqh1 (Murray *et al.* 1997). Compared to the *rqh1* null allele, *rqh1.r12* confers a number of weaker phenotypes, including reduced sensitivity to UV and ionizing radiation. In addition, the null allele is synthetically lethal with the *S. pombe rad3* and *rad26* mutations while *rqh1.r12* is not (Murray *et al.* 1997). Therefore, it is likely that DNA helicase activity is only one of the functions carried out by the RecQ homologs. Moreover, as a multifunctional helicase, Sgs1 is not unique in yeast. The Upf1 RNA helicase is required for both nonsense suppression and mRNA turnover in yeast (Czaplinski *et al.* 1998). Interestingly, inactivation of the Upf1 helicase does not cause a null phenotype; only mRNA turnover is affected. In contrast, mutations in the Upf1 N terminus affect nonsense suppression without affecting mRNA turnover (Weng *et al.* 1996).

Intragenic complementation experiments provide convincing evidence of the bipartite nature of Sgs1. These experiments confirm that the amino-terminal domain possesses an activity separate from the DNA helicase and that the two domains function in an interdependent manner. Further analysis of the enzymology of Sgs1 and its physical interactions with other proteins should shed light on this second function.

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