Purification and Characterization of the Sgs1 DNA Helicase Activity of Saccharomyces cerevisiae

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The yeast *Saccharomyces cerevisiae* Sgs1 protein is a member of a family of DNA helicases that include the *Escherichia coli* RecQ protein and the products of human Bloom's syndrome and Werner's syndrome genes. To study the enzymatic characteristics of the protein, a recombinant Sgs1 fragment (amino acids 400–1268 of the 1447-amino acid full-length protein) was overexpressed in yeast and purified to near homogeneity. The purified protein exhibits an ATPase activity in the presence of single- or double-stranded DNA. In the presence of ATP or dATP, unwinding of duplex DNA or a DNA-RNA heteroduplex by the recombinant Sgs1 fragment was readily observed. Similar to the *E. coli* RecQ helicase, displacement of the DNA strand occurs in the 3' to 5' direction with respect to the single-stranded DNA flanking the duplex. The efficiency of unwinding was found to correlate inversely with the length of the duplex region and was enhanced by the presence of *E. coli* single-stranded DNA-binding protein. In addition, the recombinant Sgs1 fragment was found to bind more tightly to a forked DNA substrate than to either single- or double-stranded DNA.

The *SGS1* gene of *Saccharomyces cerevisiae* was identified in a search for extragenic suppressors of the slow growth phenotype of cells deficient in DNA topoisomerase III, the product of the *TOP3* gene (1). Paradoxically, whereas null mutations in *SGS1* suppress the growth defect of *top3* mutants, they significantly reduce the growth rate of *top1* mutants lacking a functional DNA topoisomerase I (1–3). Based on the results of two-hybrid screens in yeast (4), it was suggested that Sgs1 protein interacted directly with DNA topoisomerase III (1) and perhaps DNA topoisomerase II as well (5). Yeast sgs1 mutants show increased genome instability; the rate of mitotic recombination between homologous sequences is elevated (1, 5, 6), and chromosome missegregation occurs more frequently during mitosis and meiosis (5, 6). These characteristics are reminiscent of those of yeast *top3* mutants lacking DNA topoisomerase III; in addition to a slow growth phenotype, *top3* mutants show an elevated frequency of recombination between repetitive sequences and are defective in sporulation (1, 7). The hyper-rec phenotype of *top3* mutants is also suppressed by mutations in *SGS1* (1).

The sequence of the Sgs1 protein suggests that it possesses a central region homologous to the *Escherichia coli* RecQ helicase (8, 9), and a helicase activity was detected in a rabbit reticulocyte coupled transcription/translation system expressing the Sgs1 protein (2). The plausible association between the yeast Sgs1 helicase and DNA topoisomerase III, a member of the type IA subfamily of DNA topoisomerases that also includes *E. coli* DNA topoisomerases I and III, is reminiscent of an enzyme termed "reverse gyrase," which was previously found only in thermophilic organisms (for a review, see Ref. 10). Reverse gyrase catalyzes positive supercoiling of DNA, and its sequence suggests that it possesses both a DNA helicase and a type IA DNA topoisomerase (Refs. 11–13; for the classification of DNA topoisomerases, see Ref. 14). In the case of the *Sulfolobus acidocaldarius* enzyme, the putative helicase and the topoisomerase activity are present on the same polypeptide (11). The association between these activities in a single enzyme led to the proposal that the enzyme acts by using its helicase activity to unwind DNA, generating both positive and negative supercoils; removal of the negative supercoils by the topoisomerase activity then results in a net accumulation of positive supercoils (11).

Recently, several additional homologues of the *E. coli* RecQ and yeast Sgs1 protein have been discovered. These include the products of the human Bloom's syndrome and Werner's syndrome genes (*BLM* and *WRN*, respectively) (15, 16). Although the clinical features of the two diseases are rather different, patients of both syndromes exhibit chromosome instability and a predisposition to cancer (for reviews, see Refs. 17 and 18). *BLM* and *WRN* proteins are similar to Sgs1 protein in size and share limited sequence homology outside of the central helicase domain, suggesting that these RecQ-type proteins might be functionally related. A *Schizosaccharomyces pombe* homologue of SGS1, termed *rgh1* , was also reported recently (19). Similar to *SGS1* of the budding yeast, *rgh1* has been shown to suppress recombination, especially during S phase arrest (19). Interestingly, the *E. coli* RecQ protein has also been shown to suppress illegitimate recombination (20).

As a first step in studying the mechanistic and functional aspects of the Sgs1 protein, we have purified a truncated form of it containing the helicase domain. We report here the DNA-dependent ATPase and helicase activities of the purified protein.

EXPERIMENTAL PROCEDURES

Plasmids for Overexpression of Sgs1 Protein and Its Truncations—The regions of the SGS1 gene encoding the N- and C-terminal segments of the protein were amplified by the polymerase chain reaction (PCR) from pRS414-SGS1 (a gift from Dr. R. Sternglanz, State University of New York at Stony Brook). The N-terminal coding segment was amplified using the VENT polymerase (New England Biolabs) and a pair of

The abbreviations used are: PCR, polymerase chain reaction; ssDNA, single-stranded DNA; Ssb, ssDNA-binding protein; ATPγS, adenosine 5’-[γ-thio]triphosphate; HA, a flu virus hemagglutinin epitope with the amino acid sequence YPYDVP.
oligodeoxynucleotides RB1 (5'-GAGTCTACCGATCAGGTTACATGGTGACGAGGCTC-3') and RB4 (5'-GGGCTTAGCTCTGCAGGAGT-3'). The sequence of RB1 was designed to place a BamHI site on GATCC (underlined) six base pairs upstream of the ATG initiation codon of SGS1, so that the final construct could be moved into a plasmid vector previously constructed for the expression of yeast DNA topoisomerase II from the inducible GAL1 gene promoter (21). The N-terminal PCR product was cloned between the BamHI and HindIII restriction sites of pBlue-Script (Stratagene) to give pRB104. The C-terminal PCR product was then introduced between the HindIII and XhoI restriction sites of pRB111 to give pRB112. The segment in pRB112 between the XhoI site and the remaining six codons of yeast DNA topoisomerase II and the remaining six codons of the N terminus of yeast DNA topoisomerase II was then replaced by the HindIII and XhoI site in the N-terminal coding region of SGS1 and the NarI site in the C-terminal coding region of SGS1. DNA specified under "Results." Recombinant Sgs1 protein were identified by SDS-polyacrylamide gel electrophoresis.

Pooled fractions were diluted with buffer A to a concentration equal to buffer A plus 0.5 mM NaCl, and imidazole was added to 5 mM. The partially purified protein solution was then mixed with nickel-agarose beads (His-Bind resin, Novagen) to allow the binding of the hexahistidine tag to the recombinant Sgs1 protein. The beads were then washed several times in buffer A containing 0.5 mM NaCl and 5 mM imidazole. After a final wash step with buffer A plus 0.5 mM NaCl and 23 mM imidazole, the recombinant Sgs1 fragment was eluted in buffer A containing 100 mM NaCl and 200 mM imidazole. The eluted protein was dialyzed against a storage buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 40% glycerol, 1 mM dithiothreitol). The final yield of the protein, estimated by the use of a protein assay kit (Bio-Rad) with bovine serum albumin solutions as standards, was between 1 and 2.5 mg for several preparations.

Nucleic Acid Substrates—DNA oligomers (GENEMED) were purified by polyacrylamide gel electrophoresis when necessary. The DNA oligomer was purchased from Dalton Chemical Laboratories. Synthetic DNA substrates were prepared from three oligonucleotides 5'-GGCGTTTCACGCGGTTACTTCGCT-3' (oligonucleotide 1), 5'-GACCTGGGAAAAGGTCGATATGACGCATTGGCAGTCTGG-3' (oligonucleotide 2), and 5'-GGCATAGACTGGTACGATATGACGCATTGGCAGTCTGG-3' (oligonucleotide 3). Oligonucleotides 2 and 3 were annealed to form the 40-base pair double-stranded DNA substrate with a one nucleotide overhang at the 5'-ends, and oligonucleotides 1 and 2 were annealed to form the forked DNA substrate with a double-stranded segment with single-stranded tails at one end (complementary regions in oligonucleotides 1 and 2 are underlined). In each case, oligonucleotide 2 was 32P-labeled at its 5'-end, using T4 polynucleotide kinase and [γ-32P]ATP. 32P-Labeled oligonucleotide 2 was also used alone as the single-stranded DNA substrate. DNA substrates for testing helicase activity were constructed by the annealing of a complementary DNA strand with single-stranded φX174 virion DNA. Short strands 26, 52, and 66 nucleotides in length were obtained by synthesis and were labeled at their 5'-ends by treatment with T4 polynucleotide kinase and [γ-32P]ATP or at the 3'-ends by treatment with terminal transferase and α32P-labeled 3'-dATP. The 52- and 66-mers were complementary to φX174 at nucleotides 130–181 and 5357–36, respectively, and the sequence of the 26-mer DNA was the same as oligonucleotide 1. The DNA strand was prepared by Whitby et al. (24). Longer strands were derived from the 140-base pair AviII-DraIII and the 558-base pair SstI-AceII restriction fragments of the double-stranded replicative form of φX174. These fragments were 32P-labeled at their 5'-ends and purified on a nondenaturing 6% polyacrylamide gel. The electroeluted fragments were then denatured and annealed with φX174 virion DNA. Conditions for DNA annealing and purification of the products were described previously (26). φX174 virion DNA and complementary DNA strand were purchased from New England Biolabs. Relaxation of supercoiled φX174 DNA by vaccinia virus topoisomerase was carried out at 37 °C in 40 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 5 mM EDTA. The relaxed DNA was phenol-extracted and ethanol-purified before use.

ATPase Assay—Reactions (20 µl each) were carried out in ATPase buffer (20 mM Tris-HCl, pH 7.5, 2 mM MgCl2, 0.1 mM ATP, 2 mM dithiothreitol, and 100 µg/ml of bovine serum albumin) for 30 min at 30 °C. Each reaction contained 25 nCi of [γ-32P]ATP and the amounts of recombinant Sgs1 protein and DNA specified under "Results." Reactions were stopped by the addition of 1 µl of 0.5 x EDTA to each, and 1 µl of each reaction was spotted onto CEL 300 PEI/UV 254 thin layer chromatography plates (Sigma). The plates were developed in 1 M formic acid and 0.5 M LiCl, and the amount of ATP hydrolyzed in the reaction mixture was evaluated from the dried plates using a phosphor imager (Fuji).

Helicase Assay—Reactions (20 µl each) contained annealed substrate DNA and were carried out as for the ATPase assay, except 2 mM MgCl2 was used. Reactions were spotted onto anling plate (5 µl of a stop buffer (0.5% proteinase K, 100 mM Tris-HCl, pH 7.5, 200 mM EDTA, 2.5% SDS) followed by incubation at 37 °C for 10 min. Products were analyzed either by electrophoresis in 1% agarose (in a buffer containing 40 mM Tris acetate, pH 8.0, 1 mM EDTA) or polyacrylamide (in 89 mM Tris borate, pH 8.3, 2 mM EDTA). Agarose gels were dried on Whatman DE81 paper, and polyacrylamide gels were dried on Whatman 3MM paper. The dried gels were analyzed using a phosphor imager.

2 R. Hanai and J. C. Wang, unpublished results.
FIG. 1. Purification of a recombinant Sgs1 protein. A, the RecQ family of DNA helicases. Protein sequences were aligned by their homologous helicase domains (shaded boxes). The number of amino acids in each of the proteins is shown to the right. B, the recombinant Sgs1 protein used in this study. The truncated protein contains amino acids 400–1268 of the full-length protein, together with an N-terminal flu virus HA tag and a C-terminal hexahistidine tag (His)6. C, SDS-polyacrylamide gel electrophoresis analysis of fractions from purification of the recombinant Sgs1. The protein was purified as described under “Experimental Procedures.” Samples were analyzed by SDS-polyacrylamide gel electrophoresis in a 6% gel, and the gel was photographed after staining with Coomassie Brilliant Blue. The position of the recombinant Sgs1 protein fragment is indicated on the right. MW, size markers; FT, flowthrough; E, eluate.

RESULTS

Purification of a Recombinant Sgs1 Protein—Initial attempts to purify full-length Sgs1 protein were unsuccessful because of the insolubility of the protein when overexpressed in S. cerevisiae. To circumvent this problem, three plasmids were constructed for the overexpression of truncated proteins from an inducible yeast GAL1 gene promoter. One plasmid was designed for expressing amino acid residues 1–1119, a second for amino acids 400–1268, and a third for amino acids 400–1447. Cells bearing the construct for the expression of Sgs1-(1–1119) yielded an insoluble protein that migrated with an apparent molecular mass of 125 and 140 kDa, respectively, with the solubility of Sgs1-(400–1268) substantially higher than that of Sgs1-(400–1447). Because of its higher solubility, the Sgs1-(400–1268) fragment was chosen for further studies. In the plasmid constructed for the expression of this fragment, pRB222, codons for MSTDPVYPYDP- (amino acids 400–1268 of Sgs1)-RRAVH6 were placed downstream of a plasmid-borne inducible promoter of the yeast GAL1 gene. Codons for two peptide motifs, a hexapeptide epitope YPYDVP of flu virus HA and a stretch of six histidines, were added to the 5' and 3' ends of the SGS1 sequence, respectively, to facilitate the detection and purification of the recombinant protein product (see “Experimental Procedures” for details). Cells of a protease-deficient S. cerevisiae strain that were transformed with pRB222. Pilot experiments showed that when the transformed cells were grown and induced with galactose, a 125-kDa protein was overexpressed (Fig. 1C, lanes b and h). Immunoblotting with an anti-HA monoclonal antibody confirmed that the overexpressed protein was the desired product (data not shown). Large scale preparations of the recombinant protein were then carried out, and the protein was purified to near homogeneity (Fig. 1C, lanes e–h).

DNA-dependent ATPase Activity—The purified recombinant Sgs1 fragment was tested for its ability to hydrolyze ATP in the presence and absence of DNA. As shown in Fig. 2, a DNA-dependent ATPase activity was readily detected. This activity was co-purified with the peak of the recombinant Sgs1 protein when eluted from a heparin column (data not shown). Hydrolysis of ATP was equally efficient in the presence of several different DNA cofactors, including either single- or double-stranded...
DNA (Fig. 2). In contrast, for the E. coli RecQ ATPase, it was previously shown that single-stranded DNA was a much more effective cofactor than double-stranded DNA (9). The slope of the beginning part of the plot shown in Fig. 2 corresponds to a rate of hydrolysis of approximately 10 ATP/s Sgs1 monomers.

DNA Binding by Sgs1—The DNA binding activity of the purified recombinant protein was investigated using the electrophoretic mobility shift assay (Fig. 3). The Sgs1 fragment was found to bind single-stranded circular dX174 DNA (ssDNA), since the presence of increasing amounts of the protein progressively reduced the gel electrophoretic mobility of the DNA (Fig. 3, lanes a–f). Binding of the protein to supercoiled dX174 appeared to be less efficient, and retardation of the DNA was observed only at the highest concentration of the recombinant protein used (lane l). The stable binding of the Sgs1 fragment to ssDNA did not require Mg(II) or nucleotide cofactors (data not shown).

To test the possibility that binding to the single-stranded DNA might be facilitated by secondary structures within it, Sgs1 binding to a synthetic duplex DNA with single-stranded tails at one end (the forked DNA substrate) was examined. The forked DNA was prepared by the annealing of two partially complementary oligonucleotides, 41 nucleotides in length. It was found that incubation of 32P-labeled forked DNA with the Sgs1 fragment resulted in the formation of a stable protein-DNA complex (Fig. 4, lanes k–o), as detected by polyacrylamide gel electrophoresis. Under the same conditions, only very weak binding to either a single-stranded oligonucleotide (lanes a–e) or duplex DNA (lanes f–j) was observed.

Helicase Activity of the Recombinant Sgs1 Protein—To test for DNA helicase activity of the recombinant protein, we examined whether it could displace a 52-nucleotide 32P-labeled oligodeoxynucleotide from single-stranded circular dX174 DNA. The Sgs1 fragment was found to effect the dissociation of the oligonucleotide from the single-stranded DNA ring in the presence of ATP and Mg(II) (Fig. 5, lanes c–g, and lanes h and i). Optimal activity occurred at a molar ratio of ATP to Mg(II) of 1:1 (data not shown). No activity was observed when ATP was replaced by the presumably nonhydrolyzable ATP analog ATPγS (lane k). Furthermore, in the presence of 2 mM ATP, the addition of 1 mM ATPγS to the reaction was found to significantly inhibit the unwinding activity (lane l). ATP could be replaced by dATP (lane m), but other common nucleotide triphosphates (e.g. GTP, TTP, and UTP) could not support DNA unwinding by the Sgs1 fragment (data not shown). These cofactor requirements are similar to those observed in the RecQ-mediated unwinding of DNA (9). Similar to the case of E. coli RecQ, the addition of Zn(II), but not Mn(II) or Ca(II), significantly inhibited the unwinding reaction (9).

A time course of the Sgs1-mediated strand displacement reaction is shown in Fig. 6. Over 50% of the labeled fragment was displaced within 5 min, and the reaction was close to completion within 10 min. The DNA unwinding activity was
The buffer contained 2 mM ATP plus 20 mM EDTA (the indicated amounts of the recombinant Sgs1 fragment. For lanes h–m). Reactions were incubated at 30 °C for 30 min in the presence of the indicated amounts of the recombinant Sgs1 fragment. For lanes i–m, the buffer contained 2 mM ATP plus 20 mM EDTA (lane i), 2 mM ATP (lane j), 1 mM ATP+SrS (lane k), 2 mM ATP plus 1 mM ATP+SrS (lane l), or 2 mM dATP (lane m). Reactions were stopped and analyzed by 1% agarose gel electrophoresis and autoradiography. A control reaction mixture was heat-denatured at 100 °C for 3 min prior to loading (lane b).

FIG. 5. DNA helicase activity of the recombinant Sgs1 fragment. Reaction mixtures each contained 1 µM DNA substrate (a 32P-labeled 52-nucleotide fragment annealed to virion φX174) in the helicase assay buffer (lanes a–g), or in the same buffer without ATP (lanes h–m). Reactions were incubated at 30 °C for 30 min in the presence of the indicated amounts of the recombinant Sgs1 fragment. For lanes i–m, the buffer contained 2 mM ATP plus 20 mM EDTA (lane i), 2 mM ATP (lane j), 1 mM ATP+SrS (lane k), 2 mM ATP plus 1 mM ATP+SrS (lane l), or 2 mM dATP (lane m). Reactions were stopped and analyzed by 1% agarose gel electrophoresis and autoradiography. A control reaction mixture was heat-denatured at 100 °C for 3 min prior to loading (lane b).

FIG. 6. Time course of the strand displacement reaction mediated by the recombinant Sgs1 protein fragment. A reaction mixture (160 µl) containing 8 µM substrate (φX174 virion DNA annealed with a 32P-labeled 52-nucleotide fragment) in the helicase assay buffer was incubated for 5 min at 30 °C prior to the addition of the Sgs1 protein fragment (0.24 nM). Following the addition of the recombinant Sgs1 fragment, 20-µl aliquots were removed at the times indicated and mixed with excess EDTA to terminate the reactions. The products were analyzed by agarose gel electrophoresis and quantitated by autoradiography.

very sensitive to the salt concentration (NaCl or potassium acetate) in the reaction buffer. There was a 50% decrease in the unwinding activity at a salt concentration between 30 and 40 mM; at 100 mM salt, the helicase activity was less than 10% of that seen in the low salt assay mixture (data not shown).

Unwinding of a DNA–RNA Hybrid—The recombinant Sgs1 fragment was also tested for its ability to unwind a DNA–RNA hybrid. A 26-nucleotide 32P-labeled RNA or DNA oligomer was annealed to φX174 virion DNA and incubated with the recombinant Sgs1 fragment in the presence of Mg(II) and ATP. Displacement of the oligoribonucleotide by the protein occurred with an efficiency similar to that of the oligodeoxynucleotide (data not shown). Like the DNA–DNA helicase activity, unwinding of the DNA–RNA hybrid was driven by the hydrolysis of ATP or dATP but not the other common nucleoside triphosphates (data not shown).

Polarity of the DNA Helicase—To determine the polarity of the helicase activity, the DNA substrates shown in Fig. 7A were used. These substrates were made by first annealing a 5′- or 3′-end-labeled oligonucleotide (66 nucleotides in length) with φX174 ssDNA. The duplex region of each was then cut by PstI restriction endonuclease to yield a linear ssDNA with either a labeled complementary 40-mer or a labeled 26-mer bound to one of its ends. The recombinant Sgs1 fragment was found to efficiently displace the 5′-labeled 40-mer (substrate I; Fig. 7B). In contrast, unwinding and dissociation of the 3′-labeled 26-mer by the protein was much less efficient (substrate II; Fig. 7B). The annealed 26-mer was not refractory to unwinding because of its sequence, since the original 66-mer was displaced with an efficiency close to that of the 40-mer (data not shown).

The Dependence of the Efficiency of Strand Displacement on the Length of the Displaced Strand—To investigate the dependence of the efficiency of DNA unwinding on the length of the duplex region, several DNA substrates were prepared using 32P-labeled fragments of 52, 140, or 558 nucleotides. As depicted in Fig. 8A, each substrate was incubated with the indicated amounts of the recombinant Sgs1 fragment, and the
products of the reaction were separated by polyacrylamide gel electrophoresis. It was found that the efficiency of strand displacement was markedly affected by its length, with more Sgs1 protein required to displace the longer DNA strands. Whereas the recombinant Sgs1 fragment displaced 60% of the 52-nucleotide fragment at a concentration of 1.2 nM (Fig. 8A, lane c, and Fig. 8B), only 50% of the 140-nucleotide fragment and 5% of the 558-nucleotide fragment was displaced at a 4-fold higher enzyme concentration of 4.8 nM (see Fig. 8A, lanes i and n, and Fig. 8B).

The effect of E. coli Ssb on the helicase activity of the recombinant Sgs1 protein was also examined. Using a DNA substrate carrying a 558-nucleotide 32P-labeled strand, it was observed that the Sgs1 helicase activity was stimulated by the presence of increasing amounts of Ssb (Fig. 9). At the highest concentration of Ssb tested (70 nM), displacement of the 558-nucleotide DNA strand was enhanced by more than 12-fold. Up to 5-fold stimulation of Sgs1 helicase activity by Ssb was also observed using a 140-nucleotide strand annealed to its single-stranded template (data not shown).

FIG. 8. Processivity of unwinding the recombinant Sgs1 fragment. DNA substrates (1 μM) containing virion φX174 DNA hybridized with 32P-labeled fragments with a length of 52, 140, or 558 nucleotides were incubated with the recombinant Sgs1 fragment in the helicase assay buffer for 30 min at 30 °C. Products were analyzed by electrophoresis in a 12% polyacrylamide gel and quantitated by autoradiography.

FIG. 9. Stimulation of the helicase activity of the recombinant Sgs1 fragment by E. coli single-stranded binding protein. Reaction mixtures (20 μl each) containing 1 μM substrate (a 32P-labeled 558-nucleotide fragment annealed to virion φX174 DNA) were incubated with Sgs1 (4.8 nM) and the indicated amounts of E. coli Ssb protein in the helicase assay buffer. Reactions were continued for 30 min at 30 °C, stopped, and analyzed by electrophoresis in a 12% polyacrylamide gel and quantitated by autoradiography.

DISCUSSION

We have shown that a purified recombinant Sgs1 fragment, which contains amino acid residues 400–1268 of the full-length protein, possesses a helicase activity with partially duplex DNA substrates. The DNA unwinding reaction is dependent on the presence of ATP or dATP. Hydrolysis of the triphosphate appears to be obligatory, as ATPγS can not substitute for ATP and inhibits the helicase activity in the presence of excess ATP. Unlike the E. coli RecQ helicase, which is stimulated by ssDNA but not double-stranded DNA, the hydrolysis of ATP by the recombinant Sgs1 protein is strongly stimulated by both forms of DNA. The polarity of unwinding is 3′ to 5′ with respect to the overhanging single-stranded DNA. The characteristics of the unwinding activity are similar to those of the E. coli RecQ protein (9), which shares homology with Sgs1 within the helicase domain. Of the other members of the RecQ family of DNA helicases (see Fig. 1A), the human RecQL protein has previously been purified and shown to have DNA unwinding activity in vitro (25, 26). Recently, the Werner’s syndrome and Bloom’s syndrome proteins were also overexpressed and shown to possess a DNA helicase activity (27–29). It thus seems likely that all of the members of the RecQ family will share a common helicase function.

The efficiency of strand displacement by the recombinant Sgs1 fragment decreases sharply with increasing length of the strand to be displaced. The unwinding assay used here, however, detects only complete displacement events, and partially unwound fragments would not be scored. It is therefore plausible that with the longer strands annealing of the DNA behind the translocating protein could reduce the observed efficiency of unwinding. Alternatively, the recombinant Sgs1 fragment might exhibit a low processivity in its reaction and might fall off the DNA before completing the displacement of the annealed fragment. The increased efficiency of unwinding of the 558-nucleotide fragment in the presence of E. coli Ssb protein is consistent with either of these interpretations.

We have also observed that Sgs1 has the ability to displace an RNA strand annealed to a longer DNA strand, with the same efficiency and cofactor requirements as displacing DNA from DNA. The product of the Werner’s syndrome gene was
also found to unwind RNA annealed to DNA (27). The ability to unwind RNA-DNA duplexes is not a common property of helicases. In one study, of four *E. coli* DNA helicases tested (30), only one (the UvR D protein) showed significant unwinding of RNA-DNA hybrids. It is not known whether the unwinding of RNA-DNA heteroduplex by the recombinant Sgs1 fragment is significant in terms of the physiological function of Sgs1 protein.

Using an assay based on the shift in the electrophoretic mobility of a DNA by bound protein, the recombinant Sgs1 protein fragment was shown to have a higher affinity for a forked DNA substrate than either single- or double-stranded DNA. This may have implications for the mechanism of DNA unwinding by Sgs1, since it has been suggested that preferential binding of the enzyme to such a junction might be important for helicase action (31). In addition, the high specificity for binding to a DNA junction could be important if the protein is to act as a decatenase in coordination with a DNA topoisomerase (see below).

The molecular mechanisms underpinning the physiological roles of the RecQ family of enzymes remain unclear. Because of the functional and physical association between yeast Sgs1 protein and DNA topoisomerase III, it was suggested that a functional and physical interaction between yeast Sgs1 and topoisomerase or its interaction with DNA. An alternative model for the action of Sgs1 protein in *in vivo* is that it might act by itself or jointly with DNA topoisomerase III in the unlinking of intertwined DNA strands. Such a decatenation activity might be involved near the end of DNA replication to resolve the intertwined parental DNA strands (1, 5, 32, 33). Failure to resolve such structures could lead to DNA strand breakage and subsequently an increase in DNA recombination and chromosome segregation. The Sgs1 protein-DNA topoisomerase III pair might also act directly on recombination intermediates to separate inadvertently paired DNA strands and thus reduce the mitotic recombination frequency (32, 33). In the case of *E. coli* DNA topoisomerase III, which resembles yeast DNA topoisomerase III in its catalytic properties *in vitro* (33, 34), the enzyme has been shown to be highly effective in the unlinking of parental strands in an *in vitro* plasmid DNA replication system (35, 36).

The identification of the Bloom’s and the Werner’s syndrome determinants as homologues of the RecQ-type proteins in *E. coli* and yeasts has greatly stimulated interest in this class of enzymes. The functional and physical interaction between the yeast Sgs1 protein and yeast DNA topoisomerase III hints that their mammalian homologues might also interact. Interestingly, recent sequencing results suggest that there are two variants of mammalian DNA topoisomerase III encoded by genes located to chromosome 17p11.2–1 (37, 38) and chromosome 22q11–12 (38). The plausible interactions between these variants, tentatively denoted DNA topoisomerases IIIα and IIIβ, and a group of helicases including the BLM, WRN, and RecQL proteins, offer challenging opportunities in their mechanistic and functional studies.

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Purification and Characterization of the Sgs1 DNA Helicase Activity of Saccharomyces cerevisiae
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