Several members of the RecQ family of DNA helicases are known to interact with DNA topoisomerase III (Top3). Here we show that the Saccharomyces cerevisiae Sgs1 and Top3 proteins physically interact in cell extracts and bind directly in vitro. Sgs1 and Top3 proteins coimmunoprecipitate from cell extracts under stringent conditions, indicating that Sgs1 and Top3 are present in a stable complex. The domain of Sgs1 which interacts with Top3 was identified by expressing Sgs1 truncations in yeast. The results indicate that the NH₂-terminal 158 amino acids of Sgs1 are sufficient for the high affinity interaction between Sgs1 and Top3. In vitro assays using purified Top3 and NH₂-terminal Sgs1 fragments demonstrate that at least part of the interaction is through direct protein-protein interactions with these 158 amino acids. Consistent with these physical data, we find that mutant phenotypes caused by a point mutation or small deletions in the Sgs1 NH₂ terminus can be suppressed by Top3 overexpression. We conclude that Sgs1 and Top3 form a tight complex in vivo and that the first 158 amino acids of Sgs1 are necessary and sufficient for this interaction. Thus, a primary role of the Sgs1 amino terminus is to mediate the Top3 interaction.

The Saccharomyces cerevisiae SGS1 gene encodes a member of the RecQ family of DNA helicases. In addition to the RecQ protein of Escherichia coli, this family includes the human BLM, WRN, RECQL4, and RECQ5 proteins as well as Rqh1 from Schizosaccharomyces pombe (1–7). These proteins play an important role in DNA metabolism as mutations in the human genes give rise to diseases characterized by genomic instability and a predisposition to cancer. Werner’s syndrome cells, which result from mutations in WRN (2), display a genomic instability termed variegated translocation mosaicism (8). Bloom’s syndrome cells, which result from mutations in BLM (1), are characterized by increased rates of sister chromatid exchange and sensitivity to DNA-damaging agents (9). Mutations in RECQL4 are found in a subset of Rothmund-Thomson syndrome cases. These cells are characterized by elevated rates of chromosomal breaks and rearrangements (5, 10). All members of this family contain a COOH-terminal domain with homology to RecQ, and all those that have been tested exhibit a 3’- to 5’-DNA helicase activity (11–15). In addition to the helicase domain, the eukaryotic proteins contain a large NH₂-terminal domain of about 650 amino acids whose sequence is poorly conserved between members. The NH₂-terminal domain is important for activity in yeast (16), but with the exception of the 3’- to 5’-exonuclease domain of WRN (17, 18) the biochemical function of the NH₂-terminal domain is unknown.

A subset of the eukaryotic RecQ family members has been shown to interact with DNA topoisomerase III (Top3) (19–22). Eukaryotic Top3 was first identified as a hyperrecombination mutant in yeast that also displayed a slow growth phenotype (23). Top3 has since been identified in several organisms including S. pombe (21, 24), Caenorhabditis elegans (25), and humans (26, 27). Like the bacterial enzyme, eukaryotic Top3 is a type I 5’-DNA topoisomerase with weak superhelical relaxing activity and a strict requirement for substrates containing single-stranded DNA or strand-passing activity (28, 29). The biological function of Top3 is unclear, but in addition to its relaxing activity E. coli topoisomerase III is notable for its ability to decatenate gapped single-stranded DNA circles (29). The recent demonstration that eukaryotic Top3 and E. coli RecQ helicase functionally interact to catenate fully duplex DNA circles (30) suggested a role for these enzymes at the termination of DNA replication to decatenate daughter chromosomes (31, 32). Although it has been suggested that RecQ helicases might function to restart stalled replication forks (7, 33–35) a role for Top3 in this process is unclear.

The SGS1 gene of yeast was identified as a mutation that suppressed the slow growth phenotype of top3 mutants (22). Thus, in contrast to top3 strains, top3 sgs1 double mutants exhibit a near wild type growth rate as well as suppression of other top3 phenotypes (22, 36). Compared with wild type cells the sgs1 single mutant displays increased rates of mitotic recombination, both at the ribosomal DNA locus and throughout the genome (22, 37), as well as increased rates of chromosome loss and missegregation (38). Like mutations in BLM, SGS1 mutations result in a hypersensitivity to methyl methanesulfonate (MMS) (16) and hydroxyurea (HU) (39).

SGS1 was cloned in a two-hybrid screen with TOP3, suggesting that Top3 interacted with the first 550 amino acids of Sgs1 (22). Because two-hybrid results do not provide evidence for direct binding, we set out to confirm this result biochemically, refine the domain of interaction, and determine whether binding was through direct protein-protein interaction. We identified an Top3-Sgs1 complex by coimmunoprecipitating and co-
fractionating these proteins from yeast extracts. The results indicate that Sgs1 and Top3 are present in a stable complex and that the NH₂-terminal 158 amino acids of Sgs1 are sufficient for complex formation. The proteins do not appear to form a simple heterodimer, however, because the full-length proteins cofractionate at a large native molecular weight. We determined that only the NH₂-terminal 158 amino acids of Sgs1 were required to bind Top3 based on an enzyme-linked immunosorbent assay (ELISA) using purified proteins. These biochemical results are consistent with our observation that phenotypes caused by mutations in the first 158 amino acids of Sgs1 can be suppressed by overexpressing Top3, whereas larger deletions cannot.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids—**Strain construction, growth, and transformation followed standard protocols (40). S. cerevisiae strain NJ7620 expresses epitope-tagged versions of Sgs1 and Top3. This strain was constructed by modifying the chromosomal SGS1 gene of wild type strain CHY125 (41) by integrating BglII-linearized plasmid pJM1526, which places three consecutive HA epitopes (YPYDVPDYA) at the NH₂ terminus of Sgs1. The gene and protein are henceforth called Sgs1-HA and Sgs1-HA, respectively. This gene was modified by integrating BglII-linearized plasmid pJM2565, which places a single V5 epitope (GPPIPPLGLDSTRTG, Invitrogen) followed by six histidines at the COOH terminus of Top3. This gene is henceforth referred to as Top3-V5 and its encoded protein as Top3-V5. Strain WYF822 was created by integrating pJM2565 into strain NJ7631 (sgs1::loxP) (16). Strain NJ7650 was constructed by deleting the SGS1 and SLY4 genes of CHY125 (41) withloxP-KAN-loxP cassettes (42) and maintaining the strain with plasmid pJM500 (SGS1/URA3). SGS1 and sgs1–34 were integrated at the LEU2 locus of NJ7650 to create strains BY1228 and BY1229, respectively. SGS1 mutant phenotypes were assayed as described (16).

Plasmid pJM1526, which expresses the epitope-tagged truncation Sgs1res1–158-HA, contains the insert from pSM105-HA (16) in the vector pRS405 (43). Plasmid pJM2565 contains a fragment of the Top3 gene encoding a COOH-terminal in-frame fusion to the V5-His₆ epitope (Invitrogen) in pRS404. To overexpress Top3 in yeast, Top3-V5 was subcloned downstream of the GALI promoter in pRS424 to make pJM2566. Plasmids expressing Sgs1-HA truncations were described (16), except for pKR1554 and pKR1555, which express epitope-tagged proteins Sgs1-HA and Sgs1res1–322-HA, respectively. To create these plasmids, the first 474 and 966 base pairs of the SGS1-HA gene were amplified by polymerase chain reaction and that the NH₂-terminal 158 amino acids of Sgs1 are sufficient for complex formation. The proteins do not appear to form a simple heterodimer, however, because the full-length proteins cofractionate at a large native molecular weight. We determined that only the NH₂-terminal 158 amino acids of Sgs1 were required to bind Top3 based on an enzyme-linked immunosorbent assay (ELISA) using purified proteins. These biochemical results are consistent with our observation that phenotypes caused by mutations in the first 158 amino acids of Sgs1 can be suppressed by overexpressing Top3, whereas larger deletions cannot.

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75 μl of PBS (10.1 mM Na₂HPO₄, 2.4 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl), pH 7.2, containing 0.1% Tween 20 (PBST) and 1 mM DTT by shaking at 60 rpm for 1 h at room temperature. After immobilization, wells were washed once with 0.4 ml of PBST plus 1 mM DTT, then blocked with 0.4 ml of 5% dried milk (w/v) in PBST plus 1 mM DTT for 1 h at room temperature. After blocking, cells were washed three times with 0.4 ml of PBST plus 1 mM DTT. A titration of 0–40 pmol of Top3 protein was added to each set of coated wells in PBST plus 1 mM DTT in a volume of 75 μl and incubated for 30 min at room temperature. After incubation with Top3-V5 protein the wells were washed three times with 0.4 ml of PBST. To detect the Top3-V5 protein, 100 μl of anti-V5 antibody (diluted 1:5,000 in PBST plus 0.5% dried milk) was added to each well for 1 h at room temperature. Wells were then washed three times with PBST, and 100 μl of anti-mouse horseradish peroxidase conjugate secondary antibody (diluted 1:5,000 in PBST plus 0.75% (w/v) dried milk) was added to each well and incubated for 1 h at room temperature. After the secondary antibody incubation, wells were washed three times with 0.4 ml of PBST, and then 200 μl of 3,3’,5,5’-tetramethylbenzidine liquid substrate system for ELISA (Sigma) was added to each well and incubated for 30 min at room temperature. After incubation, 100 μl of 0.5 N H₂SO₄ was added to each well and the absorbance of each solution read to determine the amount of Top3-V5 protein present.

RESULTS

Functional Complementation of Epitope-tagged SGS1 and TOP3—To characterize the interaction between Top3 and Sgs1, we constructed yeast strains whose chromosomal copies of the SGS1 and TOP3 genes were modified to express the COOH-terminally tagged proteins Sgs1-HA and Top3-V5 (see “Experimental Procedures”). These strains allowed us to immunoprecipitate and immunoblot the products of stable single-copy genes expressed under their native promoters. To verify that the epitope-tagged alleles behaved like wild type, we tested their ability to complement various sgs1 and top3 phenotypes. Two very sensitive measures of SGS1 and TOP3 activity are resistance to the DNA-damaging agent MMS and resistance to the DNA synthesis inhibitor HU (16). The strains expressing the tagged proteins were serially diluted and replica plated to medium containing MMS or HU. As shown in Fig. 1, the epitope-tagged strains grew as well as wild type on YPD plates and did not show the HU or MMS hypersensitivity characteristic of sgs1 or top3 strains. For example, top3 mutants grow very slowly on YPD; SGS1 TOP3-V5 cells do not display the slow growth of SGS1 top3 cells and in fact grow at the wild type rate (data not shown). Similarly, sgs1 strains grow somewhat slower than wild type, and SGS1-HA TOP3 cells grow noticeably faster than sgs1 cells. Whereas sgs1 and top3 single mutants are hypersensitive to MMS and HU (Fig. 1), the SGS1-HA and TOP3-V5 strains do not display either of these sensitivities; these strains grow like wild type in the presence of these drugs as does the SGS1-HA TOP3-V5 double-tagged strain. Based on these growth phenotypes we conclude that the epitope-tagged alleles SGS1-HA and TOP3-V5 function exactly like wild type.

Coimmunoprecipitation and Cofractionation of Sgs1 and Top3—To identify an interaction between Sgs1 and Top3, extracts were prepared from a wild type strain and from strain NJY620 expressing Sgs1-HA and Top3-V5. Following incubation of the extracts with anti-HA or anti-V5 antibodies, the immune complexes were precipitated with protein A beads and analyzed by immunoblot. Using extracts from cells expressing the tagged proteins, we observed that anti-V5 precipitated Top3-V5, as expected, and coprecipitated Sgs1-HA (Fig. 2A, lane 6). Similarly, anti-HA precipitated Sgs1-HA, as expected, and coprecipitated Top3-V5 (Fig. 2A, lane 4). These signals are specific to the epitope-tagged proteins as extract from the untagged wild type strain showed no bands of corresponding size. We note that under optimal conditions Top3-V5 coprecipitated Sgs1-HA more efficiently than Sgs1-HA coprecipitated Top3-V5 (Fig. 2A, compare lanes 2 and 4 with 6 and 8). The simplest explanation for this effect is that there is an excess of Top3 over Sgs1 protein in the extract. Such a result is consistent with the genetics of this system; lowering the Top3:Sgs1 ratio either by mutating TOP3 (22) or by overexpressing SGS1 (16) results in a profound growth defect.

The previous experiment indicates that Sgs1 and Top3 interact in cell extracts but does not address the strength of the interaction or whether these proteins require DNA to interact. We addressed these questions by varying the conditions of the immunoprecipitation from nonstringent (Buffer A plus 150 mM NaCl) to very stringent (RIPA buffer plus 50 μg/ml ethidium bromide). As shown in Fig. 2B, the intensity of the Sgs1-HA signal that coprecipitated with Top3-V5 was unaffected by changing these conditions. Likewise, the efficiency with which Top3-V5 was coprecipitated with Sgs1-HA was unaffected by changing these conditions (Fig. 2B, lower panel). Both proteins were found to coprecipitate even under the harshest conditions. We conclude that Sgs1 and Top3 are stably bound and that their interaction is not mediated by DNA.

If Sgs1 and Top3 are present in a complex then they would be expected to cofractionate over a gel filtration column. An extract from NJY620 cells was fractionated over a Superose 6 gel filtration column, and the fractions were immunoblotted to determine the elution volumes of Sgs1-HA and Top3-V5 (Fig. 3). A portion of the Sgs1-HA and Top3-V5 proteins were found to elute with a similar profile, the peak of which corresponds to a native molecular mass of ∼1.3 MDa (Fig. 3, top and middle panels). Additional Top3-V5 signal was detected in a second peak close to the void volume, although this signal was not associated with Sgs1-HA (Fig. 3, middle blot). When WFY522 (sgs1 TOP3-V5) extract was fractionated on a Superose 6 column, only the Top3-V5 signal eluting near the void volume was detected (Fig. 3, bottom panel). We conclude that the 1.3-MDa peak of Top3 is Sgs1-dependent, and the Top3-V5 signal near the void is likely to represent aggregated Top3-V5 protein that is in excess of Sgs1-HA. If a single polypeptide of Sgs1 were to interact with a single polypeptide of Top3, the expected size would be 240 kDa. The larger size of 1.3 MDa suggests that these proteins have a different stoichiometry or are complexed
with additional proteins.

**TOP3 and SGS1 Interact through the NH$_2$ Terminus of SGS1**—To determine the domain(s) of Sgs1 responsible for interaction with Top3, strain WYF823 (TOP3-V5 sgs1::loxP) was transformed with a series of plasmids expressing Sgs1-HA truncations under the control of the native SGS1 promoter (16). Extracts were prepared and IPs performed under RIPA conditions. A fragment of Sgs1 consisting of amino acids 1–652 (Sgs1$_{1-652}$-HA) coprecipitated with Top3-V5 (Fig. 4A), consistent with a Top3 interaction domain in the NH$_2$-terminal 550 amino acids as determined by the two-hybrid assay (22). In contrast, no interaction was detected between Top3-V5 and the DNA helicase domain of Sgs1 (Sgs1$_{1-158}$-HA) (Fig. 4B). These results indicate that the interaction between Sgs1 and Top3 is mediated through the NH$_2$ terminus of Sgs1. To map the interaction domain more accurately, fragments of Sgs1-HA containing all but the NH$_2$-terminal 158 or 322 amino acids were expressed in bacteria and purified on glutathione beads. As shown in Fig. 5A, unfused GST protein and GST-Sgs1$_{1-158}$-HA were highly purified, whereas GST-Sgs1$_{1-158}$-HA contained several smaller bands that are likely to be breakdown products because their abundance varied between preparations. Recombinant full-length Top3-V5 was highly purified using Ni-affinity chromatography (Fig. 5A). ELISA wells were coated with 15 pmol of purified GST, GST-Sgs1$_{1-158}$-HA, or GST-Sgs1$_{1-322}$-HA and nonspecific sites blocked with 5% dried milk. Increasing amounts of purified Top3-V5 protein were then incubated in a series of wells prior to washing and detecting bound Top3-V5 with anti-V5 antibody and a chromogenic substrate. This assay revealed weak background binding of Top3-V5 to unfused GST protein that saturated at 30 pmol of input Top3-V5 (Fig. 5B). In contrast, both GST-Sgs1$_{1-158}$-HA and GST-Sgs1$_{1-254}$-HA bound increasing amounts of Top3-V5 protein. At the highest input level of Top3-V5, these Sgs1 domains bound three times more Top3-V5 than GST alone. This result demonstrates a direct protein-protein interaction between the amino terminus of Sgs1 and Top3. Little difference between GST-Sgs1$_{1-158}$-HA and GST-Sgs1$_{1-322}$-HA was detected, confirming that the first 158 amino acids contain a significant portion of the interacting domain.

**Top3 Overexpression Complements Mutations in the NH$_2$ Terminus of Sgs1**—We previously used a synthetic lethal screen to identify several novel "SLX" mutants that require SGS1 for viability (41). Phenotypically, sgs1Δ and slx4Δ single mutants are viable, but the sgs1Δ slx4Δ double mutant is dead. Because Sgs1 activity is essential for viability in this background, slx4Δ mutants provide a genetic system to identify functional domains of SGS1. Structure-function analysis previously revealed that small NH$_2$-terminal deletions or mutations in the DNA helicase domain of Sgs1 were lethal (16).

To address the question of why NH$_2$-terminal deletions of Sgs1 were defective in this assay, we tested whether overexpression of Top3 could rescue the synthetic lethal phenotype. The starting strain, NJY560 (slx4Δ sgs1Δ pJM500, SGS1/URA3), is nonviable on medium containing the drug 5-FOA because it selects against the SGS1/URA3 plasmid, which is essential for viability in this background. NJY560 was first transformed with a plasmid expressing the TOP3 gene under control of the inducible GAL1 promoter (pJM2566, GAL1p-TOP3) and then with a series of SGS1 deletions in a LEU2 vector. In contrast to the LEU2 vector alone, wild type SGS1 allowed these cells to grow on 5-FOA (Fig. 6A). Complementation of the synthetic lethal phenotype by SGS1 is independent of Top3 overexpression because growth is observed under both repressed (glucose) and induced (galactose) conditions. As ex-
expected, a helicase-defective allele of SGS1 (sgs1-hd) and all NH2-terminal truncations of Sgs1 were lethal when streaked onto 5-FOA plates containing glucose (Fig. 6A). In contrast, when these strains were streaked on 5-FOA galactose, the sgs1-DN50 and sgs1-DN158 alleles displayed complementing activity. Neither sgs1-hd nor sgs1-DN322 complemented, even when Top3 was overexpressed. These results indicate that deletion of amino acids 1–158 significantly impairs the interaction between Top3 and Sgs1, and increasing the Top3 concentration restores this interaction. Based on this assay we conclude that the size of the interaction domain in vivo must be larger than amino acids 1–158 and smaller than 1–322.

The sgs1–34 mutation was isolated as a temperature-sensitive allele of SGS1 caused by the amino acid change Q31P. At the restrictive temperature (37 °C) sgs1–34 behaves like sgs1-DN158, suggesting that it lacks Sgs1 NH2-terminal function.2 As a result of this mutation, strain BSY1229 (sgs1–34 slx4) is viable at 25 °C but not at 37 °C. We tested whether Top3 overexpression could suppress this NH2-terminal mutation. Strain BSY1229 was transformed with pJM2566 (GAL1p-TOP3) and the transformants streaked on selective plates containing glucose or galactose at 25 or 37 °C (Fig. 6B). When Top3

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expression was repressed by growth on glucose plates, the strain grew at 25 °C but not at 37 °C. However, when Top3 was overexpressed by growth in the presence of galactose the strain was able to grow at 37 °C (Fig. 6B). The suppression of sgs1–34 by Top3 overexpression is allele-specific, as two other SGS1 temperature-sensitive alleles whose mutations map to the DNA helicase domain could not be suppressed (data not shown). As above, we conclude that the sgs1–34 mutation impairs the binding of Top3 to Sgs1 at the restrictive temperature and that increasing Top3 concentration restores the interaction.

Post-translational Modification of Sgs1—We suspected that the failure to identify a strong Sgs1-Top3 interaction by in vitro translation might be due to the requirement for an in vivo modification to Sgs1. We addressed this question by asking whether Sgs1 is phosphorylated in vivo. Yeast cells expressing Sgs1-HA or a control HA-tagged protein were grown in the presence of [32P]PO4. Extracts were prepared from these strains, and proteins were immunoprecipitated and analyzed by SDS-PAGE and autoradiography. As shown in Fig. 7, immunoprecipitation of Sgs1-HA results in a [32P]-labeled band migrating at 220 kDa, as expected for Sgs1-HA. The 220-kDa band is specific to Sgs1-HA as only the expected 100-kDa protein is precipitated with anti-HA from a control extract. An additional control revealed that only the expected 69-kDa RPA1 protein was immunoprecipitated from the Sgs1-HA extract with an antiserum to RPA1. Taken together, these data indicate that phosphorylated forms of Sgs1 and RPA1 do not interact under these conditions. We conclude that Sgs1 is phosphorylated during exponential growth in vivo.

DISCUSSION

Although a DNA fragment encoding the amino-terminal 550 amino acids of Sgs1 was isolated in a two-hybrid screen using Top3 as bait (22), there has been no biochemical confirmation of this interaction or any evidence of a direct interaction between these two proteins. To address the in vivo association of these proteins we created epitope-tagged alleles of SGS1 and TOP3 which were stably integrated at their chromosomal locations and expressed under their native promoters. These alleles were active in all of the biological assays we examined, indicating that the tagged proteins retain wild type function. Our data show that Sgs1 and Top3 can be coimmunoprecipitated under stringent buffer conditions including 0.1% SDS and ethidium bromide. These results are consistent with the idea that these proteins are present in a stable complex in vivo.

As summarized in Fig. 8, our deletion analysis indicates that a domain as small as the NH2-terminal 158 amino acids of Sgs1 is able to bind to Top3 in vivo. ELISAs using highly purified proteins provide biochemical evidence that Top3 binds Sgs11–158 through direct protein-protein interactions. As expected for such a physical interaction, Top3 overexpression suppressed phenotypes associated with mutations in the Sgs1 amino terminus. The fact that overexpressed Top3 suppressed Sgs1 with NH2-terminal deletions as large as 158 amino acids suggests that the Top3 interaction domain extends further than residues 1–158 in vivo (Fig. 8). Because overexpressed Top3 failed to suppress a deletion of 322 amino acids, we conclude that the Top3 interaction domain in vivo is larger than amino acids 1–158 and smaller than 1–322.

Recent evidence suggests that Top3 interacts with some, but not all, RecQ family members. Bacterial Top3 and RecQ were shown to interact functionally in vitro to catenate double-stranded DNA circles (30). In S. pombe, the top3+ gene has been identified as an essential gene whose lethal phenotype is suppressed by mutations in rqh1+, the S. pombe RecQ homolog (21). In human cells, immunolocalization studies indicate that BLM is present in promyelocytic leukemia nuclear bodies together with Top9a (19, 50). The human RecQ5β protein was also shown to colocalize and coimmunoprecipitate with Top3 (4). In contrast, there is as yet no evidence that WRN interacts with Top3, although it is associated with a large complex of replication proteins including topoisomerase I (51).

Recently, Wu et al. (20) used a far Western blotting technique to show that human Top3β bound BLM by direct protein-protein interactions. These experiments demonstrated that the NH2-terminal 212 amino acids of BLM was sufficient for binding Top3. Our observation that Top3 binds a corresponding region of Sgs1 (1–158) suggests that the NH2 termini of BLM and Sgs1 are functionally conserved. A comparison of the Sgs1
null phenotypes (16). Considering that the phenotypes produce “hypermorphic” phenotypes that are more extreme than the sgs1-phenotype, it is inconsistent with the fact that Sgs1 fragments inhibit Top3 activity in vitro (52) and that sgs1-D158 produces growth defects even in a top3 background (16). An alternative model to explain these results is that a third factor interacts with Top3 and Sgs1. Deletions of the Sgs1 NH$_2$-terminal 158 amino acids might result in growth defects by reducing binding to Top3 as well as this yet to be identified third factor.

The Top3-Sgs1 complex isolated from yeast is resistant to RIPA buffer, suggesting that the interaction is very stable. This affinity is retained even in a complex between Top3 and the relatively small Sgs1$_{1-158}$-HA fragment (Fig. 4). Given this apparent high affinity, it is surprising that multiple studies have required very sensitive methods to detect interactions between Top3 and RecQ helicases in vitro. We required a very sensitive ELISA, and it was reported that a Top3-Sgs1 complex formed in vitro was dissociated by the relatively gentle conditions of 140–250 mM NaCl (52). As mentioned above, chemical cross-linking was used to detect an interaction between Top3 and BLM (20). This suggests that the Top3-helicase complexes formed in vitro are different from those formed in vivo. This difference in affinity may simply reflect suboptimal binding conditions in vitro; higher protein concentrations and/or co-translation might be required to form a stable complex. Alternatively, the correct interaction between Top3 and Sgs1 might depend on other cellular factors or modifications. As a test of this we found that Sgs1 is phosphorylated. The biological function of the phosphorylation is unknown, but it might regulate Sgs1 DNA helicase activity or the interaction of Sgs1 with Top3 or other proteins.

The Top3-Sgs1 complex eluted from a Superose 6 column at an approximate size of 1.3 MDa, indicating that it exists in a multimeric complex. Although we cannot rule out the possibility that a small amount of DNA mediates this complex, we feel it is unlikely for the following reasons. First, high speed extracts were used to remove bulk chromatin. Second, Top3 and Sgs1 coimmunoprecipitated despite treatment with ethidium bromide, which has been found to disrupt protein-DNA interactions. Third, we have examined the elution of double-stranded DNA by Superose 6 chromatography and found that DNA larger than 4 kilobases elutes in the void, whereas 1-kilobase DNA fragments elute at ~2 MDa. Thus, if the complex at 1.3 MDa were mediated by DNA, the fragments would have to be very small and discreet in size.

If the 1.3-MDa complex is a multimer, it might consist of a hexamer of Top3 with a hexamer of Sgs1 which would be expected to run at that size. Other helicases have been shown to exist as hexamers, such as E. coli DnAB (55) and more significantly, human BLM (56). Alternatively, other proteins might be present in the complex as reported recently for BLM. In addition to its presence in promyelocytic leukemia bodies, BLM is associated with a number of human DNA repair proteins in the BASC complex including some that are conserved in yeast (50, 57, 58). Based on the interactions of BLM in human cells, it is possible that Sgs1 and Top3 are associated with additional proteins that contribute to its large native molecular weight and stable association. Purification of the Top3-Sgs1 complex will be required to determine which of these models is correct.

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