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The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs

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ABSTRACT

The *Saccharomyces cerevisiae* Sgs1p helicase localizes to the nucleolus and is required to maintain the integrity of the rDNA repeats. Sgs1p is a member of the RecQ DNA helicase family, which also includes *Schizosaccharomyces pombe Rqh1*, and the human *BLM* and *WRN* genes. These genes encode proteins which are essential to maintenance of genomic integrity and which share a highly conserved helicase domain. Here we show that recombinant Sgs1p helicase efficiently unwinds guanine–guanine (G-G) paired DNA. Unwinding of G-G paired DNA is ATP- and Mg^{2+}-dependent and requires a short 3′ single-stranded tail. Strikingly, Sgs1p unwinds G-G paired substrates more efficiently than duplex DNAs, as measured either in direct assays or by competition experiments. Sgs1p efficiently unwinds G-G paired telomeric sequences, suggesting that one function of Sgs1p may be to prevent telomere–telomere interactions which can lead to chromosome non-disjunction. The rDNA is G-rich and has considerable potential for G-G pairing. Diminished ability to unwind G-G paired regions may also explain the deleterious effect of mutation of *Sgs1* on rDNA stability, and the accelerated aging characteristic of yeast strains that lack Sgs1 as well as humans deficient in the related WRN helicase.

INTRODUCTION

The *Saccharomyces cerevisiae SGS1* gene encodes a helicase that is essential for maintaining genomic stability. The protein product of *SGS1*, Sgs1p, localizes to the nucleus and is particularly concentrated within the nucleolus (1), where ribosomal DNA (rDNA) transcription and rRNA processing occur (2,3). Mutation of *SGS1* results in defective chromosome segregation, and increased mitotic and illegitimate recombination (4–7). Consistent with the nucleolar localization of Sgs1p, *SGS1* is critical to maintenance of the rDNA repeats. *Sgs1*-deficient cells are characterized by increased rDNA recombination (4,5) and accumulation of extrachromosomal rDNA circles containing one or more rDNA repeats (6).

Sgs1p belongs to a DNA helicase family of which the prototypical member is *Escherichia coli* RecQ (8, reviewed in 9,10). Among the other eukaryotic helicases in this family are *Schizosaccharomyces pombe* Rqh1p (11); the human BLM helicase, deficient in Bloom's syndrome (12); and the human WRN helicase, deficient in Werner's syndrome (13). Mutations in *E.coli RecQ*, *S.pombe Rqh1*, and the human WRN and *BLM* genes all result in hyperrecombination and genomic instability; and the human genetic diseases, Bloom's syndrome and Werner's syndrome, are associated with frequent development of malignancies (11,14–18). Like Sgs1p (1), WRN localizes to the nucleolus in rapidly dividing cells (19,20).

Sgs1p (21), BLM (22) and WRN (23–25) are all ATP-dependent helicases which unwind duplex DNA with 3′-5′ directionality *in vitro*. The RecQ family helicases are to some extent functional homologs, as either *BLM* or *WRN* can suppress the hyperrecombination characteristic of *Sgs1* mutant yeast (7). Moreover, yeast strains deficient in Sgs1p exhibit premature aging that may be analogous to the premature aging characteristic of the human genetic disease, Werner's syndrome (1).

Eukaryotic cells contain a variety of helicases active on duplex DNA, RNA–DNA hybrids and RNA (reviewed in 26,27). It is of considerable interest to understand why, despite the presence of these many helicases, the RecQ family helicases are of such unique importance to genomic stability. Specific interactions with other proteins are likely to contribute to the critical functions of Sgs1p in genomic stability. The *SGS1* gene was identified initially as an extragenic suppressor of the slow growth phenotype characteristic of yeast deficient in topoisomerase III, and the *SGS1* protein product, Sgs1p, was shown to interact directly with topoisomerase III (4). Yeast strains deficient in *top3* display pleiotropic effects including DNA hyperrecombination and chromosomal missegregation (4,28,29). Sgs1p also interacts with topoisomerase II (29), a topoisomerase essential for faithful chromosome segregation during mitosis and meiosis (30,31).

Substrate specificity is also a critical determinant of helicase function. We have recently demonstrated that recombinant BLM helicase (rBLM) can unwind DNAs in which guanine–guanine (G-G) pairing stabilizes interstrand interactions (32). These experiments used as model substrates G4 DNA, in which G-G pairing stabilizes interactions between four DNA strands. The BLM helicase was shown to unwind G4 DNA 10–20-fold more efficiently than duplex substrates, as measured both in direct unwinding assays and in competition experiments. Furthermore, G-G paired substrates are not unwound by at least one other very

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potent helicase, *E.coli* RecBCD. These results suggested that G-G paired DNAs may be natural substrates of BLM and related helicases *in vivo*, and that failure to unwind G-G paired DNAs may cause or contribute to the genomic instability characteristic of cells deficient in RecQ family helicases.

We have now asked whether *S.cerevisiae* Sgs1p can unwind G-G paired DNA. We have studied the activity of recombinant protein (amino acid residues 400–1268; 21), which carries the central helicase domain that is highly conserved among RecQ family members. Here we report that, like the related BLM helicase, Sgs1p not only unwinds G-G paired DNA substrates but is considerably more active on G-G paired than on standard duplex substrates. We show that Sgs1p unwinds G-G paired structures formed from G-rich telomeric repeats, raising the possibility that impaired ability to disrupt telomere–telomere interactions may contribute to the chromosome non-disjunction characteristic of *Sgs1* mutants. The *S.cerevisiae* rDNA consists of several hundred tandem repeats of a 1.3 kb transcription unit. The rDNA repeats are G-rich on the top (non-template) strand and therefore have considerable potential to form G-G paired structures. A critical role for Sgs1p in unwinding G-G paired rDNA may cause or contribute to the dramatic effect of mutation of *SGS1* on rDNA stability, which results in premature aging (1,6). Analogous activity of the WRN helicase may contribute to accelerated aging characteristic of Werner's syndrome.

MATERIALS AND METHODS

Formation of G-DNA and duplex DNA substrates

The following deoxyoligonucleotides were used in this study: Scc-T, AACTTGTGTGGGTGTGTGTGGGTGTGTGT; Scc, AACTTGTGTGGGTGTGTGTGGG; TP, TGGACCAGACCTAGCAGCTATGGGGGAGCTGGGGAAGGTGGGAATGTGA; OX-1T, ACTGTCGTACTTGATATTTTGGGGTTTTGGGGAATGTGA; OX-1, ACTGTCGTACTTGATATTTTGGGGTTTTGGGG; H1, GCATCGGCTTCCCAACTAGCTTTTTTTTTT; K1, TTTTTTTTTTTTTTTTTTTTTTTTTTTGCTAGTTGGGAAGCCGATGC.

Formation of G4 DNA and G2′ DNA from TP, OX-1 and OX-1T, and duplex DNA by annealing H1 and K1, was performed as described (32,33). Formation of G-G paired DNAs from Scc and Scc-T was carried out by incubating oligonucleotides at 2–3 mg/ml in TE (10 mM Tris–HCl, pH 7.4, 1 mM EDTA) containing 1 M NaCl for G4 DNA formation (34) or 1 M KCl for G2′ DNA formation (35) at 37°C for 48 h. After incubation, samples were diluted 1:5 with 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, 12.5 mM KCl and 2.5% glycerol, and DNAs resolved on a 8% non-denaturing polyacrylamide gel (29:1 polyacrylamide: bisacrylamide) run in 0.5× TBE (50 mM Tris–borate, pH 8.2, 0.5 mM EDTA) containing 10 mM KCl at 4°C, at 5–8 V/cm. Bands corresponding to G4 DNA, G2′ DNA and single-stranded DNA (ssDNA) were identified according to their relative mobility by UV-shadowing or autoradiography and excised. DNAs were eluted from the crushed gel slices by soaking in TE containing 50 mM NaCl and 20 mM KCl at room temperature for 8–12 h, precipitated with ethanol, washed, and stored at –20°C. DNA was labeled as described (32), and G-G pairing was verified by assaying characteristic protection of the guanine N7 from methylation with dimethylsulfate (DMS) (36).

Methylation interference and protection

For methylation interference assays, 5′ end-labeled single-stranded oligonucleotides were methylated with DMS prior to G4 DNA formation. Samples were first heated at 94°C for 3 min, chilled on ice to ensure complete denaturation, then incubated with 0.1% DMS in buffer containing 50 mM sodium cacodylate, pH 7.0, 1 mM EDTA for 15 min at room temperature. DNA was ethanol precipitated, washed with ethanol, used to form G4 DNA and/or G2′ DNA as described above, purified, precipitated, resuspended in 100 µl of 1.0 M piperidine, heated at 90°C for 30 min, then lyophilized, washed with 25 µl deionized water twice, resuspended in formamide loading dye and resolved on a 16% polyacrylamide, 7 M urea denaturing gel. For methylation protection, gel-purified G4 DNA, G2′ DNA or ssDNA were treated with DMS and resolved by electrophoresis on an 8% acrylamide gel in 0.5× TBE, 10 mM KCl. Bands were excised and DNAs eluted, precipitated, and cleaved with 1.0 M piperidine at 90°C for 30 min. Samples were then resolved by 16% denaturing polyacrylamide gel electrophoresis.

Helicase assays

Truncated recombinant Sgs1 protein carrying the central conserved helicase domain (residues 400–1268 of the full-length 1447 residue polypeptide) was over-expressed in *S.cerevisiae* and purified as described (21). Helicase activity was assayed by monitoring unwinding of G-G paired or duplex DNA substrates to single-stranded oligonucleotides. Reactions were carried out in buffer containing 50 mM Tris–HCl, pH 7.4, 2 mM $MgCl_2$, 2 mM ATP, 50 mM NaCl and 100 µg/ml BSA at 37°C for 20 min (unless otherwise indicated), terminated by addition of SDS/proteinase K to final concentrations of 0.5% and 0.5 mg/ml, respectively, and incubated at 37°C for 15 min longer. Samples were resolved on 8% non-denaturing-polyacrylamide gels in 0.5× TBE, 10 mM KCl. Gels were dried and exposed for autoradiography or scanned and quantitated by a phosphoimager. For assays of unwinding kinetics, DNA was equilibrated in the reaction buffer at 37°C for 10 min, and the reaction was initiated by addition of Sgs1p and terminated by addition of EDTA to a final concentration of 10 mM. The quantities of Sgs1p and DNA in each experiment are specified in the figure legends.

RESULTS

Formation of G-G paired DNA substrates

Guanine-guanine interactions (37) can promote the association of DNA or RNA strands which contain runs of three or more consecutive guanine residues (34,36,38). The structures that can be produced by G-G pairing are distinguished by strand stoichiometry, strand orientation and conformation of glycosidic bonds, and include four-stranded parallel G4 DNA or antiparallel two-stranded G2′ DNA (Fig. 1A and B; reviewed in 39). In G-G paired DNA, the ring N7 bonds with the exocyclic amino group of a neighboring guanine, and is inaccessible to methylation, so G-G pairing can be readily verified by DMS probing (36). In this study, several different synthetic deoxyoligonucleotides, including Scc, Scc-T and OX-1T, were used to form both G4 DNA and G2′ DNA, and G-G interactions were confirmed by DMS modification (Fig. 1C).

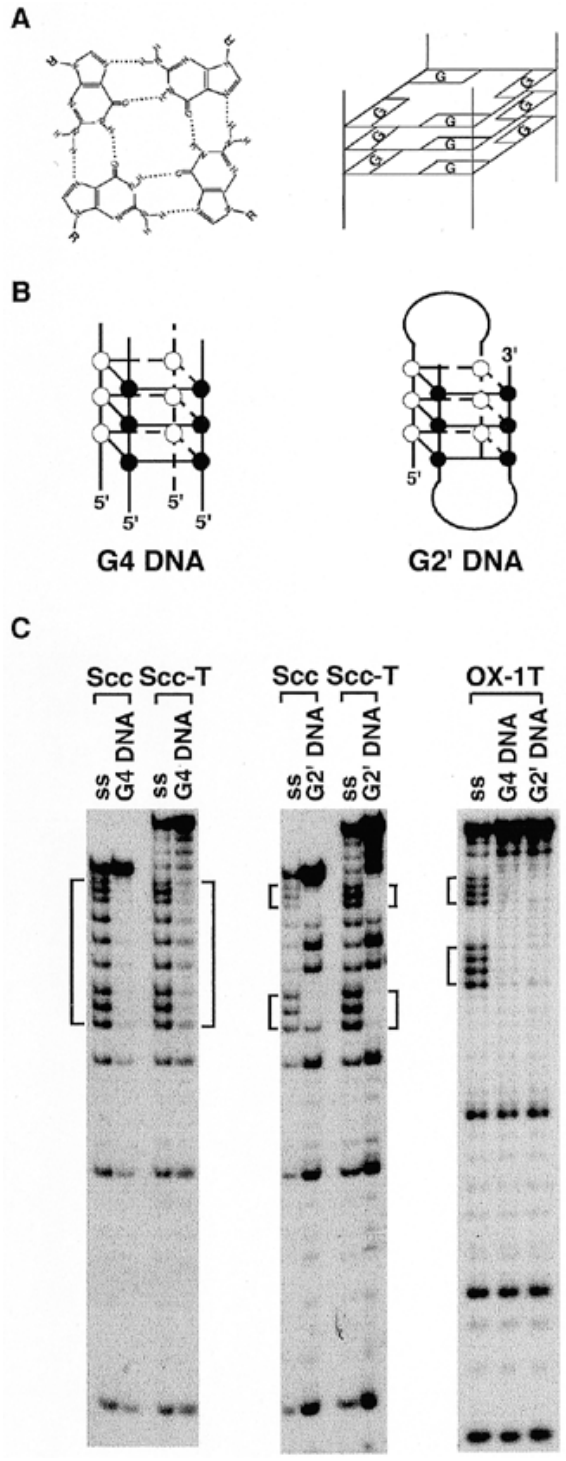

Figure 1. G-G paired DNAs. (**A**) Guanine residues interact via Hoogsteen pairing to form a G-quartet, a cyclic planar array (left); strand association mediated by G-G pairing is further stabilized by stacking of G-quartets (right). (**B**) G-G paired DNA as four-stranded parallel G4 DNA (left) and as an antiparallel hairpin dimer, G2′ DNA (right). (**C**) Methylation interference analysis of ^{32}P-labeled G4 DNA formed from oligonucleotides Scc and Scc-T (left); methylation protection of G2′ DNA prepared from oligonucleotides Scc and Scc-T (center); and methylation protection of G4 DNA and G2′ DNA prepared from oligonucleotide OX-1T (right). The guanine residues involved in G-G Hoogsteen pairing are bracketed.

Sgs1p unwinds G4 DNA

To ask if Sgs1p can unwind G-G paired DNA, purified Sgs1p was incubated with G4 DNA formed from the Scc-T oligonucleotide, which carries $TG_{1–3}$ repeats like those found in *S.cerevisiae* telomeres (reviewed in 40), and unwinding was monitored by non-denaturing polyacrylamide gel electrophoresis. Figure 2A shows that the fraction of G4 DNA unwound increased with time, and complete unwinding was evident by 10 min. This time course is similar to that of G4 DNA unwinding catalyzed by rBLM helicase (32).

Sgs1p is a 3′ to 5′ DNA helicase that requires a short 3′ single-stranded region to function on duplex DNA (21). We compared unwinding of two G4 DNA substrates, one formed from Scc-T, which has a 7 nt 3′ tail, and the other formed from Scc, which has a blunt 3′ end. The tailed substrate, G4 -Scc-T, was completely unwound during 20 min incubation with Sgs1p at a 1:100 molar ratio of Sgs1p helicase to G4 DNA, while there was no unwinding of the blunt substrate G4-Scc even at 40-fold higher enzyme:DNA ratios (Fig. 2B). The dependence of unwinding upon a 3′ single-stranded tail is also a characteristic of rBLM helicase (32).

Both Mg^{2+} and ATP were required for G4 DNA unwinding, and the non-hydrolyzable ATP analog, ATPγS, did not support the unwinding reaction (Fig. 2C). No unwinding occurred when both ATPγS and ATP were present. ATPγS also inhibits Sgs1p unwinding of duplex DNA in the presence of ATP (21). The most straightforward explanation for this observation is that ATPγS is a competitive inhibitor with higher affinity for Sgs1p than ATP. Analyses of other helicases have found that non-hydrolyzable nucleotides may have higher affinities for the enzyme than hydrolyzable analogs (27).

We also assayed unwinding activity of Sgs1p on several additional G4 DNAs, G4-TP, G4-OX-1T and G4-OX-1. Both OX-1T and OX-1 carry two repeats of T_4G_4, the telomeric sequence of the ciliate, *Oxytricha*. G4 DNA formed from either will have a 5′ single-stranded region, but only G4-OX-1T will have a free 3′ single-stranded tail. Sgs1p unwinds G4-TP and G4-OX-1T, but not G4-OX-1, which lacks a 3′ tail (Fig. 2D). These observations further confirm that Sgs1p is a 3′–5′ helicase which requires a short single-stranded 3′ tail for activity (see also Fig. 2A) (21). They also demonstrate that G4 DNA unwinding is independent of DNA primary sequence.

Sgs1p unwinds G2′ DNA

When G-G paired DNA is formed in the presence of K^+ rather than Na^+ as the major monovalent cation, the predominant products are hairpin dimers in which the two strands are antiparallel, referred to as G2′ DNA (33,34,41) (Fig. 1B). As in G4 DNA, the guanines in G2′ DNA display characteristic protection from DMS probing (Fig. 1C), but G2′ DNA migrates significantly faster than G4 DNA on non-denaturing gel electrophoresis. The minor species (asterisks in Fig. 3) in the preparation is likely to be due to formation of an isomer in which adjacent G-G paired strands adopt a different glycosidic conformation, as has been reported by others (41).

Kinetic analysis of Sgs1p unwinding of G2′ DNA substrates formed from oligonucleotide OX-1T showed that the fraction of G2′ DNA unwound increased linearly with time, and complete unwinding was evident by 7 min (Fig. 3A). This time course is similar to that of G4 DNA unwinding by Sgs1p (Fig. 2A).

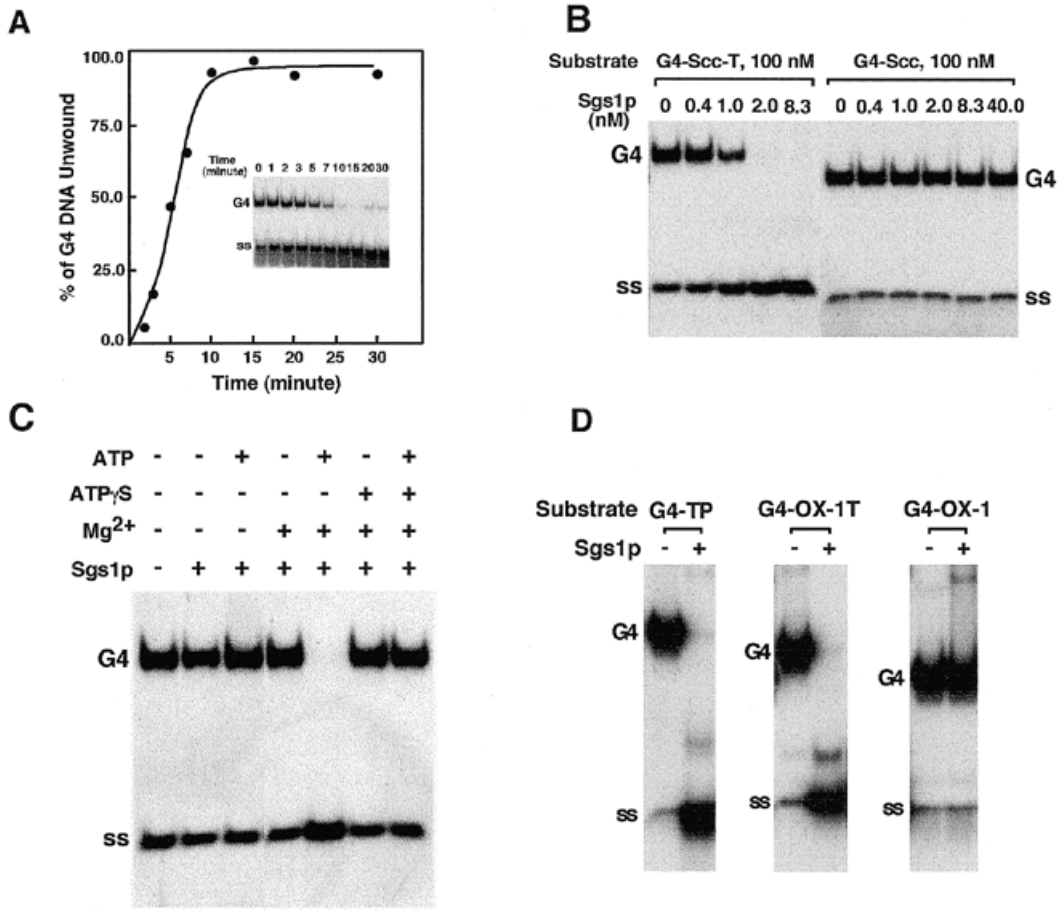

Figure 2. Unwinding of G4 DNA by Sgs1p. (**A**) Kinetics of G4 DNA unwinding. ^{32}P-labeled G4 DNA formed from the Scc-T oligonucleotide (100 nM) was incubated with Sgs1p (8 nM) for the times indicated, and samples resolved on an 8% non-denaturing gel (inset). The graph shows the fraction of G4 DNA unwound at each time. Mobilities of G4 DNA and ssDNA are indicated in each panel. (**B**) Sgs1p-dependence of G4 DNA unwinding. ^{32}P-labeled G4 DNA (100 nM) formed from the Scc-T or Scc oligonucleotides was incubated with the indicated amount of Sgs1p and products analyzed by gel electrophoresis. (**C**) G4 DNA unwinding activity requires Mg^{2+} and ATP. ^{32}P-labeled G4 DNA (100 nM) formed from the Scc-T oligonucleotide was incubated with Sgs1p (8 nM) in the presence or absence of 2 mM ATP, 2 mM ATPγS or 2 mM Mg^{2+}, as indicated. (**D**) 3′–5′ helicase activity of Sgs1p on G4-DNA substrates. Unwinding assays of Sgs1p (0.4 nM) on ^{32}P-labeled G4 DNA substrates (2.5 nM) prepared from oligonucleotides TP, OX-1T and OX-1.

Complete unwinding was evident at a protein:DNA molar ratio of 1:20 (Fig. 3B, left). G2′ DNA formed from the oligonucleotide OX-1, which lacks a single-stranded tail, was not unwound by Sgs1p (Fig. 3B, right). Like duplex (21) and G4 DNA unwinding (Fig. 2C), G2′ DNA unwinding required Mg^{2+} and ATP, and was not supported by ATPγS (Fig. 3C).

G-G paired DNA is a better substrate for Sgs1p than duplex DNA

We compared Sgs1p unwinding of G-G paired DNA and standard Watson–Crick duplex DNA in both direct assays and competition experiments, using as substrate a synthetic duplex ‘fork’ with single-stranded 3′ and 5′ tails generated by annealing oligonucleotides H1 and K1. Sgs1p did not completely unwind the fork substrate, even at protein:DNA ratios greater than 1:1 (Fig. 4A). Sgs1p-catalyzed unwinding of the H1/K1 fork substrate is therefore considerably less efficient than unwinding of G-G paired DNAs (compare Figs 2B and 3B). The preference for G-G paired substrates was further established in competition experiments, in which unwinding of radiolabeled H1/K1 duplex fork or TP-G4 DNA was assayed in the presence of unlabeled competitor. These experiments showed that unwinding of TP-G4 DNA was diminished 75% in the presence of a 5-fold molar excess of TP-G4 DNA, and abolished at 10-fold molar excess; but that the H1/K1 duplex fork did not compete for TP-G4 DNA unwinding even at a 20-fold molar excess (Fig. 4B). Conversely, unwinding of the H1/K1 duplex fork was diminished ~75% in the presence of a 1-fold molar excess of TP-G4 DNA, and abolished at 2-fold molar excess (Fig. 4C). These results establish that Sgs1p preferentially unwinds G-G paired DNA.

DISCUSSION

We have shown that purified recombinant Sgs1p helicase unwinds G-G paired DNA. Like Sgs1p unwinding of duplex DNA (21), Sgs1p unwinding of G-G paired DNA requires a 3′ single-stranded tail and is dependent upon the presence of Mg^{2+} and ATP in the reaction. Strikingly, Sgs1p unwinds G-G paired substrates at least 10-fold more efficiently than it unwinds duplex substrates. This suggests that G-G paired DNAs may be natural targets for the Sgs1p helicase *in vivo*.

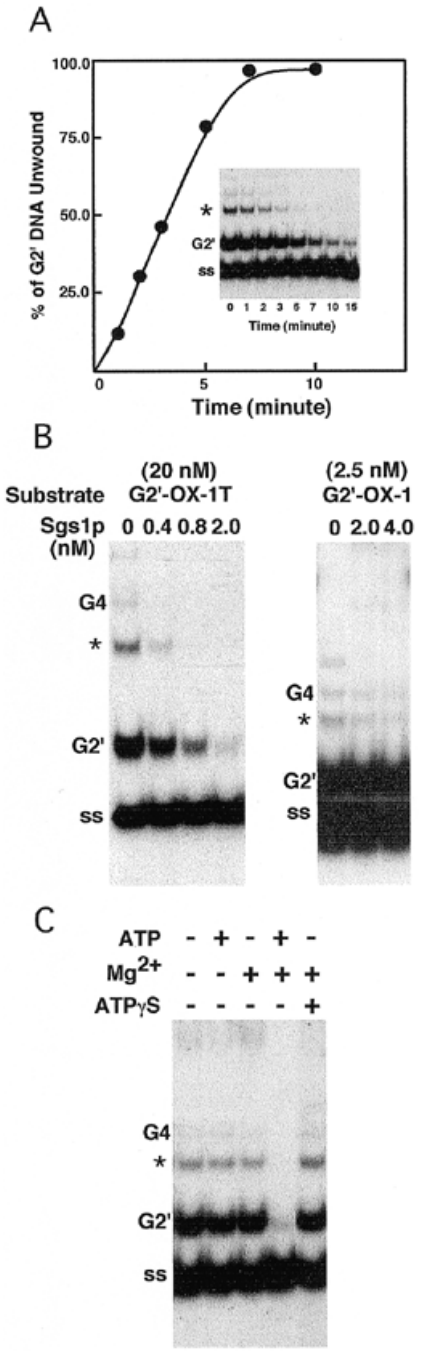

Figure 3. Unwinding of G2′ DNA by Sgs1p. (**A**) Kinetics of G2′ DNA unwinding. ^{32}P-labeled G2′ DNA prepared from the OX-1T oligonucleotide (50 nM) was incubated with Sgs1p (4 nM) for the times indicated, and samples resolved on an 8% non-denaturing gel (inset). The graph shows the fraction of G2′ DNA unwound at each time. Mobilities of G4 DNA, G2′ DNA and ssDNA are indicated in each panel. (**B**) Sgs1p-dependence of G2′ DNA unwinding. ^{32}P-labeled G2′ DNAs prepared from oligonucleotides OX-1T (20 nM) or OX-1 (2.5 nM) were incubated with the indicated amount of Sgs1p and products analyzed by gel electrophoresis. (**C**) Unwinding requires Mg^{2+} and ATP. ^{32}P-labeled G2′ DNA prepared from the OX-1T oligonucleotide (10 nM) was incubated with Sgs1p (4 nM) in the presence or absence of 2 mM ATP, 2 mM ATPγS or 2 mM Mg^{2+}, as indicated. A species that migrates between G4 DNA and G2′ DNA on the gel is indicated by an asterisk.

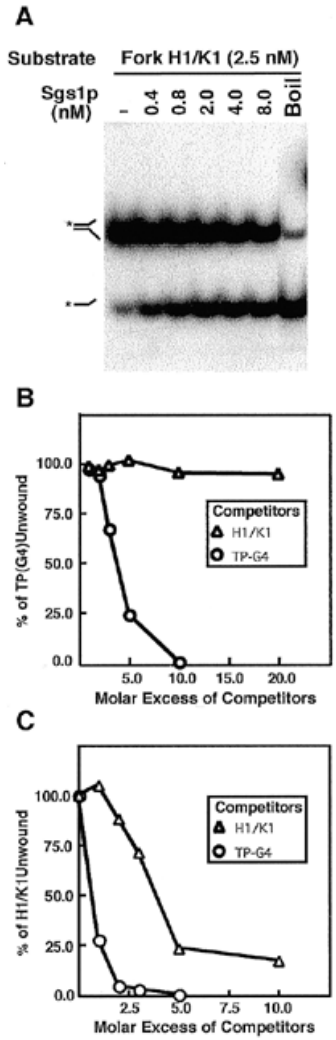

Figure 4. Sgs1p preferentially unwinds G4 DNA. (**A**) Sgs1p activity on duplex DNA substrate. ^{32}P-labeled H1/K1 duplex fork DNA substrate (2.5 nM) was incubated with the indicated amount of Sgs1p and products analyzed by gel electrophoresis. Mobilities of the duplex fork substrate and the single-stranded unwinding product are indicated at the left. (**B**) Competition assay of unwinding of ^{32}P-labeled G4 DNA formed from the TP oligonucleotide in the presence of unlabeled G4-TP or H1/K1 duplex. Unwinding products were resolved by gel electrophoresis, the fraction of unwound substrate quantitated by phosphoimager and graphed as percentage of maximal unwinding. (**C**) Competition assay of unwinding of ^{32}P-labeled H1/K1 duplex fork DNA substrate in the presence of unlabeled G4-TP or H1/K1 duplex. Quantification as in (B).

Enzymes of the RecQ helicase family have drawn considerable attention because they play key roles in maintaining genomic stability (8–10). *SGS1* appears to be the only *RecQ* family gene in *S.cerevisiae*. Other RecQ family helicases that have been identified in eukaryotic cells include *S.pombe* Rqh1 and the human BLM and WRN helicases (11–13). These proteins share seven conserved helicase motifs but differ in their C- and N-termini (9,10). There appears to be unusual conservation of function within this gene family, because either *BLM* or *WRN* can suppress the hyperrecombination phenotype of yeast *Sgs1* mutants (7).

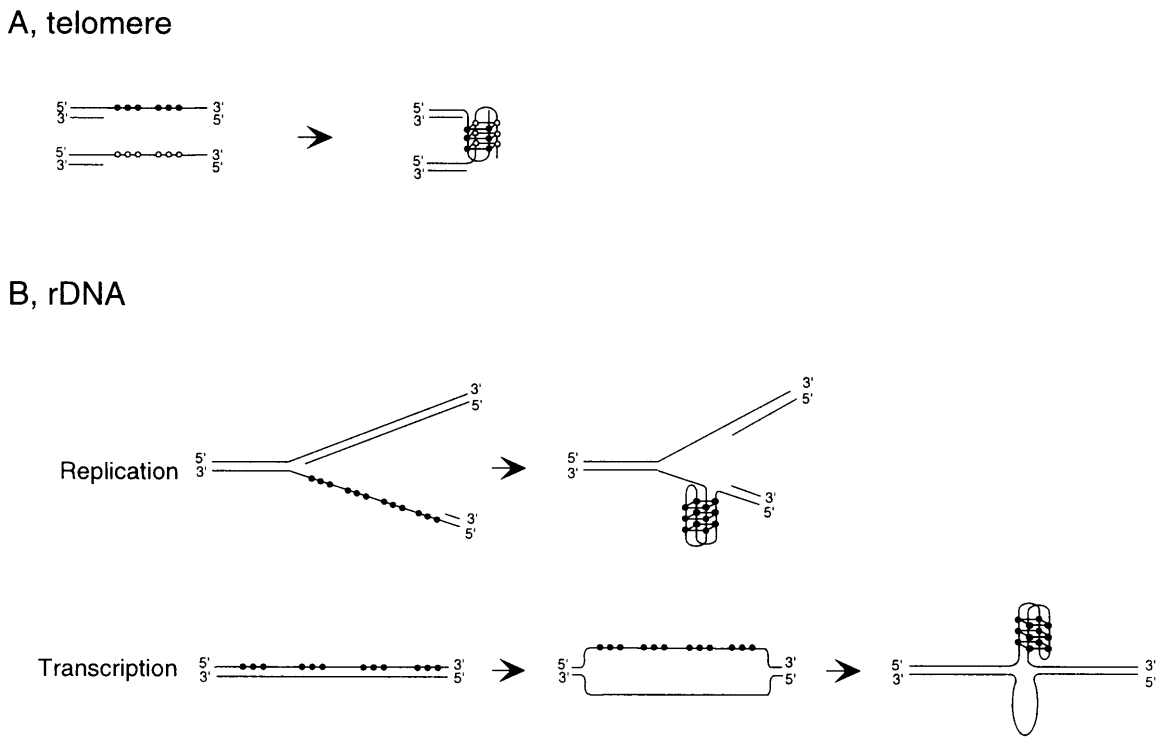

Figure 5. A model for formation of G-G paired DNA structures in rDNA and telomeres. (**A**) Telomeres. In essentially all eukaryotic chromosomes, the G-rich strand of the telomere extends to form a short 3′ tail which is the primer for telomerase. Intermolecular G-G pairing could result in chromosomal non-disjunction. (**B**) rDNA. The eukaryotic rDNA contains one G-rich strand, which has the potential to form non-Watson–Crick structures stabilized by G-G pairing. The figure shows how such structure might form during rDNA replication (above) or transcription (below). Open and closed circles denote guanines. Diagrams of G-G paired structures show for simplicity interactions involving guanines that are closely spaced. Formation of G-G paired structures *in vivo* could involve DNA spanning hundreds of nucleotides or more.

The Sgs1p preparation used in these experiments was a truncated fragment containing amino acids 400–1268 of the wild-type 1447 residue polypeptide (21). We used truncated Sgs1p in part because it is difficult to purify intact, full-length Sgs1p at yields useful for detailed biochemical analysis. In addition, we have recently shown that, like Sgs1p, recombinant human BLM helicase preferentially unwinds G-G paired DNAs (32). These results raised the question of whether ability to unwind G-G paired DNAs might be a general property of the eukaryotic RecQ helicases. Truncated Sgs1p contains the central conserved helicase domains. As truncated Sgs1p and full-length rBLM behave similarly in unwinding G-G paired DNA substrates, the determinants necessary for recognition and unwinding of G-G paired DNA therefore reside within the central region containing the helicase domains. This central region is highly conserved among RecQ family helicases, so it is likely that other helicases of this family, including the WRN helicase deficient in Werner's syndrome, will prove to unwind G-G paired substrates.

Formation of alternative, non-Watson–Crick structures is thought to contribute to genomic instability in the expansions of triplet repeats that can lead to a variety of neurological deficiencies (42 and citations therein). G-rich DNA has an analogous potential to form alternative structures stabilized by G-G pairing during cellular processes that unwind the DNA duplex, including transcription, recombination or replication. ssDNAs which contain runs of three or more consecutive guanine residues readily self-associate *in vitro* to form structures stabilized by G-G pairing (33,34,38,43–46). That G-G interactions occur spontaneously in solution was first established nearly 40 years ago, when concentrated solutions of GMP were found to form a gel upon storage at 4°C (47). For the experiments described in this manuscript, high yields of G4 DNA were produced by incubation of synthetic oligonucleotides at 60°C for 48 h, but synthetic oligonucleotides form G4 DNA spontaneously under normal storage conditions; typically, preparations of synthetic oligonucleotides which have G runs in their sequences will contain a few percent G4 DNA. G-G paired structures are very stable once formed. For example, the T_m of the TP oligonucleotide as duplex DNA is <75°C, while the T_m of G4-TP is >90°C (34). Preferential unwinding of a G4 DNA substrate is therefore unlikely to reflect relative thermodynamic stabilities of G4 and duplex DNA. Although G-G paired DNA has not been directly observed *in vivo*, one reason may be that cells contain helicases, such as Sgs1p and BLM, which are very active on G-G paired substrates.

In yeast, two chromosomal domains are notably G-rich, the telomeres and the rDNA. (In mammalian cells, the immunoglobulin heavy chain switch regions comprise a third G-rich chromosomal domain.) We suggest that formation of G-G paired structures may cause or contribute to the genomic instability and hyperrecombination phenotypes characteristic of cells lacking RecQ family helicases. Mechanisms by which this may occur in *S.cerevisiae* are outlined in Figure 5, and discussed below with respect to the phenotypes of *Sgs1* mutants.

Essentially all eukaryotic telomeric repeats contain one G-rich strand which extends to form a short tail at the 3′ end of telomere

and which primes telomere extension by telomerase (reviewed in 48–52). Intermolecular G-G pairing between the 3′ tails of different telomeres may result in chromosomal non-disjunction in the absence of an appropriate unwinding activity (Fig. 5A). We have shown that Sgs1p can unwind G-G paired structures formed by telomeric repeats. The absence of this activity could result in the chromosomal non-disjunction characteristic of strains deficient in Sgs1p (29).

The rDNA of essentially all eukaryotes is G-rich on the non-template strand and has considerable potential for G-G pairing. In *S.cerevisiae*, mutation of *SGS1* destabilizes the rDNA repeats (1,4,5), which results in premature aging (6). While the complementary strand in the Watson–Crick duplex would normally protect the G-rich strand from intrastrand G-G pairing, the duplex is transiently denatured during replication and transcription (Fig. 5B). The denatured G-rich strand may then form G-G paired structures which are normally unwound by Sgs1, but which in the absence of Sgs1 unwinding activity must be eliminated by recombination. This would account for the accumulation of rDNA circles in *Sgs1* mutant strains. The nucleolar localization of Sgs1p (1) is also consistent with preferential activity of Sgs1p on the G-rich rDNA.

RecQ family helicases are highly conserved within the helicase domain. The human WRN helicase, which is a member of this family, localizes to the nucleolus in rapidly dividing cells (19,20). Our observations that both BLM (32) and Sgs1 actively unwind G-G paired DNAs raise the interesting possibility that impaired G-G unwinding activity of WRN helicase could contribute to or cause the accelerated aging characteristic of Werner's syndrome.

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