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Sgs1 Regulates Gene Conversion Tract Lengths and Crossovers Independently of Its Helicase Activity†

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RecQ helicases maintain genome stability and suppress tumors in higher eukaryotes through roles in replication and DNA repair. The yeast RecQ homolog Sgs1 interacts with Top3 topoisomerase and Rmi1. In vitro, Sgs1 binds to and branch migrates Holliday junctions (HJs) and the human RecQ homolog BLM, with Top3α, resolves synthetic double HJs in a noncrossover sense. Sgs1 suppresses crossovers during the homologous recombination (HR) repair of DNA double-strand breaks (DSBs). Crossovers are associated with long gene conversion tracts, suggesting a model in which Sgs1 helicase catalyzes reverse branch migration and convergence of double HJs for noncrossover resolution by Top3. Consistent with this model, we show that allelic crossovers and gene conversion tract lengths are increased in *sgs1*Δ. However, crossover and tract length suppression was independent of Sgs1 helicase activity, which argues against helicase-dependent HJ convergence. HJs may converge passively by a "random walk," and Sgs1 may play a structural role in stimulating Top3-dependent resolution. In addition to the new helicase-independent functions for Sgs1 in crossover and tract length control, we define three new helicase-dependent functions, including the suppression of chromosome loss, chromosome missegregation, and synthetic lethality in *srs2*Δ. We propose that Sgs1 has helicase-dependent functions in replication and helicase-independent functions in DSB repair by HR.

The repair of DNA double-strand breaks (DSBs) by homologous recombination (HR) is critical for maintaining genome stability and cancer suppression. DSBs are produced by ionizing radiation, genotoxic chemicals, and nucleases and when replication forks encounter DNA damage. Broken ends are converted to Rad51 nucleoprotein filaments that search for and invade homologous duplex DNA, producing a Holliday junction (HJ) intermediate. Branch migration of HJs extends or eliminates heteroduplex DNA (hDNA), and mismatches in hDNA are repaired, resulting in a region of localized loss of heterozygosity termed a gene conversion tract.

Crossovers accompany some gene conversions, posing risks of deletions, inversions, translocations, and large-scale loss of heterozygosity (31, 45, 54). The mechanisms that suppress tract lengths and crossovers are important to elucidate because they determine the extent and frequency of the loss of heterozygosity during DSB repair by HR and thereby regulate genome stability.

In the yeast *Saccharomyces cerevisiae*, mitotic and meiotic crossovers are suppressed by Sgs1 (26, 55), a member of the RecQ helicase family that includes five human proteins, three of which (BLM, WRN, and RECQ4) suppress tumors (25). RecQ helicases have conserved structures and interactions with type I topoisomerases (e.g., yeast Top3). Yeast Rmi1 is in complex with Sgs1-Top3 and may promote binding to branched DNAs or Top3 strand passage (10, 43). Yeast *sgs1*, *top3*, and *rmi1* mutants show DNA damage hypersensitivity, genome instability, slow growth, poor sporulation, and hyperrecombination (10, 43). Sgs1-Top3-Rmi1 has roles in processing HR intermediates, restarting blocked or collapsed replication forks, and activating S-phase checkpoint arrest (5, 6, 8, 10, 13, 15, 16, 19, 26, 32, 43, 48, 55). These roles in replication are thought to underlie the synthetic lethality/sickness of *sgs1* with *srs2*, *rrm3*, *slx1*, *slx4*, *mus81*, and *mms4* mutations (15, 17, 58, 64). The synthetic lethality/sickness of *sgs1*Δ *srs2*Δ mutants is suppressed by defects in *RAD51* and other HR genes, suggesting that Sgs1 and Srs2 play redundant roles in resolving recombination intermediates that arise during replication (15). *sgs1*Δ cells are hypersensitive to DNA-damaging agents like methyl methanesulfonate (MMS) and to replication stress induced by hydroxyurea (7, 16, 40, 56), and they show enhanced rates of spontaneous HR, gross chromosomal rearrangements, and chromosome loss (3, 20, 44, 68). Cells lacking Sgs1 or Srs2 frequently arrest as large-budded cells with a single nucleus in the mother cell or "stuck" between mother and daughter cells (37). Thus, Sgs1 has important roles in both DNA repair and replication.

Crossover suppression by Sgs1 suggests that this protein promotes HJ resolution by a noncrossover mechanism, and this suggestion is supported by biochemical evidence. RecQ, Sgs1, and BLM each display DNA binding and ATP-dependent helicase activity and have a strong affinity for four-way junctions that resemble HJs (5, 24, 28, 74). Moreover, RecQ, Sgs1, and BLM branch migrate HJs (5, 24, 28) and BLM-Top3α resolves a synthetic double-HJ substrate by "dissolving" HJs into one another via reverse branch migration; this pro-

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duces a hemicatenane that is unlinked by Top3α (74). HJ branch migration by BLM and the dissolution of double HJs by BLM-Top3α are both ATP dependent, presumably reflecting a requirement for BLM helicase activity (28, 74). Although the Sgs1 unwinding of duplex DNA requires ATP and a functional helicase/ATPase domain (6, 34), ATP/helicase dependencies of Sgs1 in HJ branch migration have not been reported. A reversed replication fork resembles an HJ, hence Sgs1-Top3-Rmi1 may process related branched DNA structures that arise at blocked replication forks and during DSB repair by HR (39).

Ira et al. (26) suggested that Sgs1 suppresses crossovers by reverse branch migrating double HJs until they converge and are resolved without crossing over by Top3. This model makes two testable predictions. First, in the absence of Sgs1, double HJs would be free to forward branch migrate, extending hDNA and increasing conversion tract lengths. This prediction is consistent with more frequent association of crossovers with long conversion tracts (27, 62). Second, reverse branch migration should require a functional Sgs1 helicase, therefore tract lengths should also be increased in helicase-defective mutants. Consistent with the first prediction, we confirmed that crossovers are increased in *sgs1*Δ and show that allelic gene conversion tract lengths are increased. Surprisingly, these phenotypes were suppressed by helicase-defective Sgs1, thus defining two new helicase-independent roles for Sgs1. We also define three new helicase-dependent roles for Sgs1 in suppressing DSB-induced chromosome loss, chromosome missegregation, and synthetic lethality in *srs2*Δ. These results are discussed in relation to Sgs1 function in replication and HR-mediated DSB repair.

MATERIALS AND METHODS

Yeast strains. All strains are derivatives of YPH250 (59) (see Table S1 in the supplemental material). *sgs1*Δ was replaced with *hisG* as described previously (4). A *KanMX* cassette was used to create *srs2*Δ (52) and *top3*Δ (this study). Helicase-defective *sgs1* alleles were created at the natural *SGS1* locus as follows. An 1,874-bp fragment from the central region of *SGS1* was amplified by using primers 5′-CGGGATCCAATCAAATAGGCGTGGAGCA-3′ and 5′-CCGCTCGAGAAGGTGTCATGGTTAGCCTGA-3′, digested with BamHI and XhoI, and inserted into the BamHI and SalI sites of pUC19 carrying *URA3*. Lysine 706 was converted to arginine (*sgs1KR*) or alanine (*sgs1KA*) with primers 5′-GCCAACAGGGGGCGGCCGTTCTCTTTGCTATC-3′ and 5′-AACAGGGGGTGGCGCCTCTCTTTGCTATC-3′, respectively (14); these create new EagI and NarI sites for mapping mutations. These vectors were used to replace *SGS1* with *sgs1KR* and *sgs1KA* by two-step replacement (9). Mutations were confirmed by Southern hybridization and complete *sgs1* sequencing. Sgs1, Sgs1KA, and Sgs1KR expression levels were measured by Western blot analysis using anti-Sgs1 antibodies (yT-18; Santa Cruz Biotechnology). Strains carry a galactose-inducible source of HO nuclease (*GALHO*) to create DSBs in one copy of *ura3* and *MAT***a**-inc and/or *MAT*α-inc mutations to prevent *MAT* cleavage. Synthetic lethality was monitored by standard meiotic spore analysis.

HR assays. DSB-induced allelic HR frequencies were measured using nonselective assays as described previously (47). Briefly, *GALHO* was induced for 6 h in liquid culture before cells were seeded to yeast extract-peptone-dextrose (YPD) plates and HR products were screened by replica plating YPD colonies to appropriate selective media. HR frequencies were calculated as the number of recombinants per YPD colony; typically, 1,000 to 1,500 colonies were scored in each of four determinations per strain. Broken chromosomes have a telomere-proximal *HIS3* gene (*HIS3*:telV), and chromosome loss was scored directly among Ura^- His^- products as described previously (33). Crossovers can produce His^- and an associated product with two copies of *HIS3*:telV (His^{++}), which was identified by PCR among at least 40 His^+ products per strain. His^- products can arise by break-induced replication (BIR), which comprises nonloss/noncrossover His^- products (47). Crossover frequencies were estimated as nonloss, non-BIR His^- frequencies and by His^{++} frequencies. Gene conversion tract spectra and average minimum tract lengths were determined as described previously (47). Analogous procedures were used to analyze direct repeat HR (11). All statistical analysis was performed with *t* tests unless noted otherwise.

Analysis of DSB-dependent cell killing, MMS sensitivity, and nuclear segregation. DSB-dependent cell killing was calculated as the relative plating efficiencies on YPD for cultures incubated in YP-galactose–YPD for 6 h during HR assays. To determine MMS sensitivity, exponential-phase YPD cultures were diluted and seeded in parallel on YPD and YPD-0.01% MMS plates and incubated at 30°C for 5 days before colonies were scored. Relative survival was calculated as the ratio of colonies arising in the presence or absence of MMS. Nuclear segregation was analyzed in exponential-phase cultures (10^7 cells/ml), treated with 0.1% MMS or mock treated, and incubated at 30°C. Aliquots were removed at specified times, MMS was inactivated by the addition of 10% sodium thiosulfate, and cells were immediately fixed in methanol:glacial acetic acid (3:1 vol/vol) for 1 to 24 h at 4°C, mounted with mounting medium containing 1.5 μg/ml DAPI (4′,6′-diamidino-2-phenylindole), and examined by fluorescence microscopy with a Zeiss Axioskop 2 microscope.

RESULTS

Sgs1 regulates gene conversion tract lengths. To determine whether Sgs1 suppresses mitotic gene conversion tract lengths, we employed a system in which allelic HR at *ura3* is initiated by an HO-induced DSB flanked by 11 phenotypically silent mutations that create restriction fragment length polymorphisms (RFLPs) spanning a 3.5-kbp region; the donor locus carries a frameshift mutation (X764) located 332 bp downstream of the HO site in the recipient allele (Fig. 1A) (47). Coconversion of the HO site and X764 yields Ura^- recombinants, and the fraction of Ura^- recombinants provides an estimate of the gene conversion tract lengths. We also estimated tract lengths by determining the conversion frequencies of all of the RFLP markers; this determination also gives detailed information about tract directionality and continuity. In some cases, we mapped just the two most distant markers flanking the DSB (called R5′ and B3′). This system also provides measures of HR efficiency, crossovers, chromosome loss, BIR, and DSB-dependent cell death. We studied *sgs1*Δ and mutants expressing helicase-defective Sgs1KA or Sgs1KR from the normal chromosome XIII locus. A fraction of DSBs created by HO are repaired by precise rejoining, and there is less HO cleavage of the *ura3* site than at *MAT* (12), probably because access at *ura3* is limited by nucleosomes, whereas the HO site at *MAT* is nucleosome-free (70). Typically, <20% of cells undergo HR during a 6-h *GALHO* induction.

Spontaneous HR is increased in *sgs1*Δ (20, 68), but DSB-induced allelic HR levels were similar in wild-type (WT) and *sgs1*Δ cells (Fig. 1B). HO cleavage is similar in wild-type and *sgs1*Δ cells (26), indicating that Sgs1 does not have a major role in regulating the efficiency of HR repair of HO-induced DSBs. Because crossovers are increased in *sgs1*Δ (26, 55) and are more frequently associated with long gene conversion tracts (27, 62), we predicted that tract lengths would increase in *sgs1*Δ and this was indeed the case. The fraction of long-tract (Ura^-) conversions increased from 67% in the wild type to 88% in *sgs1*Δ. Consistent with prior reports, crossovers were increased in *sgs1*Δ (Fig. 2A and B). High-resolution mapping of 90 HR products from *sgs1*Δ revealed increased conversion frequencies for most markers flanking the DSB (see below) and an increase in average minimum tract length from 1.7 kbp in the wild type to 2.8 kbp in *sgs1*Δ. Other than increased tract length, the *sgs1*Δ conversion tract spectrum was similar to that of the wild type

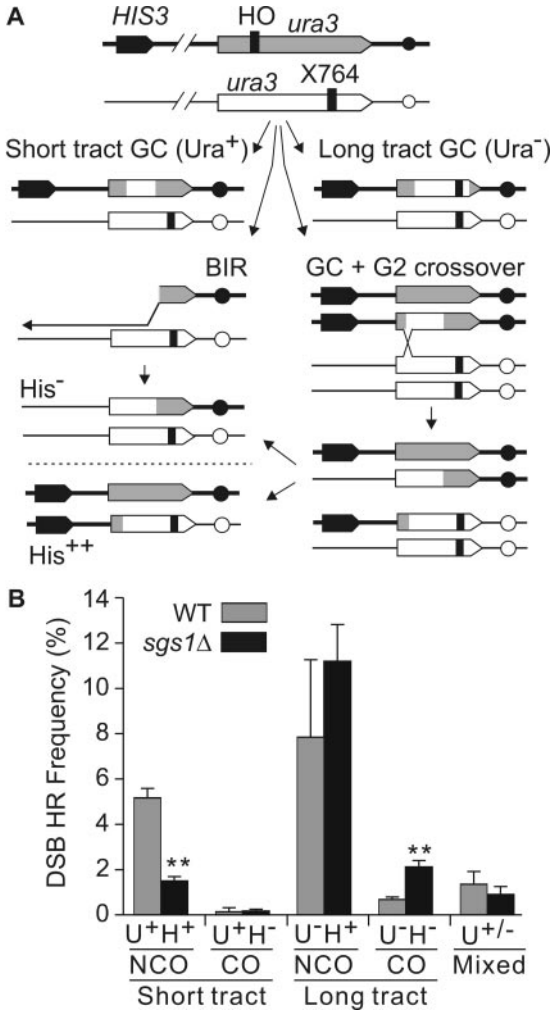

FIG. 1. DSB-induced allelic HR in WT and *sgs1*Δ cells. (A) Fates of DSBs in allelic HR substrate. The parent structure is shown at the top, with shading in the recipient *ura3* allele indicating silent RFLP markers (see Fig. S1 in the supplemental material). DSBs are created at the HO recognition site. The HO site and X764 (black bars) are inactivating, frameshift mutations. *HIS3* is near the telomere, 110 kbp from *ura3*. Short- and long-tract gene conversions (GC) without crossovers are predominant in wild-type cells. Crossovers in G_2 lead to the gain/loss of *HIS3*, producing His^- and His^{++} products in 50% of subsequent mitotic divisions; the other 50% remain heterozygous at *HIS3*. BIR leads only to the His^- product shown above the dashed line. Loss of the broken chromosome produces Ura^- His^- products (not shown). (B) DSB-induced HR frequencies, defined as the number of products per YPD colony scored. The remaining colonies were mostly parental but included rare BIR and chromosome loss events (Fig. 5). Values are averages ± standard deviations (SD) (error bars) for four determinations. Short- and long-tract gene conversions include His^+ (mostly noncrossover [NCO]) and His^- (crossover [CO]) products. His^- values exclude BIR and chromosome loss products. $Ura^{+/-}$ includes sectored colonies of several types with different His phenotypes; most $Ura^{+/-}$ colonies arise from independent HR events in G_2 cells (47). Significant differences ($P < 0.01$) are indicated by double asterisks. Increased fractions of Ura^- products in *sgs1*Δ indicate longer tract lengths, and increased fractions of His^- (H^-) products indicate increased crossovers.

(Table 1 and see Fig. S1 in the supplemental material). The increased tract lengths and crossovers in *sgs1*Δ are not specific to the densely marked HR substrate as similar results were obtained in strain JC3666, which has only the X764, R5′, and

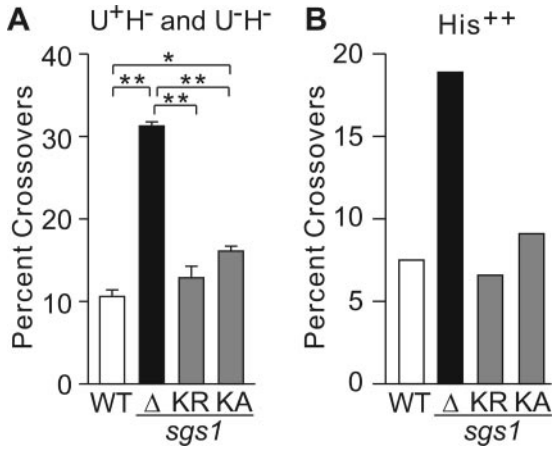

FIG. 2. Sgs1 regulation of crossovers is helicase independent. (A) Percentage of His^- crossovers including short- (Ura^+) and long-tract (Ura^-) HR products. Values are averages ± SD (error bars) for four determinations. Because only 50% of crossovers become His^-, these crossover values are twice the raw values in Fig. 1B. The double asterisks indicate significant differences at a P value of <0.0001; the single asterisk indicates a P value of 0.0015. All other comparisons showed no significant differences ($P > 0.05$). These values exclude His^- products arising by chromosome loss or BIR. (B) Percentage of His^{++} crossover products ($n \geq 40$ per strain).

B3′ markers flanking the HO site (data not shown). Thus, Sgs1 does not strongly affect the efficiency or fidelity of DSB repair by HR but it does regulate outcome, including gene conversion tract lengths and crossovers.

Sgs1 helicase-independent regulation of conversion tract lengths and crossovers. By analogy to the Rad3 ATPase (61), a mutation of the conserved Walker ATPase lysine (K706 in Sgs1) to arginine (Sgs1KR) should allow ATP binding but not hydrolysis and a mutation to alanine (Sgs1KA) should prevent ATP binding. Because helicases require ATP hydrolysis to perform work, reverse branch migration of HJs should require a functional helicase. We therefore expected that Sgs1KR would not promote HJ reverse branch migration or suppress crossovers and tract lengths. Surprisingly, the *sgs1KR* mutant displayed wild-type crossover levels (Fig. 2) and wild-type tract lengths measured by conversion frequencies of X764, R5′, and B3′ (Fig. 3). In the *sgs1KA* mutant, tract lengths were also similar to that of the wild type (Fig. 3), and crossovers, measured as fractions of His^- products, were slightly above wild-type levels but significantly lower than *sgs1*Δ levels (Fig. 2A). We also measured crossovers as fractions of His^+ products that were His^{++}, and these results confirmed that Sgs1KR and Sgs1KA suppress crossovers to essentially wild-type levels (Fig. 2B). Thus, Sgs1 suppresses tract lengths and crossovers in vivo

TABLE 1. Gene conversion tract distributions (%) in the wild type and *sgs1*Δ[a]

Genotype	Bidirectional	HO only	5′	3′	Discontinuous
SGS1	73	0	17	2	8
*sgs1*Δ	92	0.4	4	0	8

[a] Data for the wild type are from 75 products (for details, see reference 47). Data for *sgs1*Δ are from 90 products.

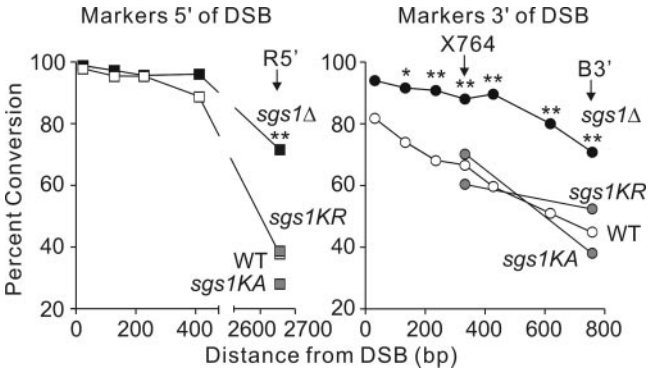

FIG. 3. Sgs1 regulation of gene conversion tract lengths is helicase independent. Conversion frequencies for individual markers, 5′ or 3′ of the DSB, are indicated for the wild-type, *sgs1*Δ, *sgs1KR*, and *sgs1KA* strains. See Fig. S1 in the supplemental material for data for the wild type and *sgs1*Δ. X764 conversion frequencies were determined as fractions of Ura^- colonies among an average of 5,600 colonies scored per strain. R5′ and B3′ conversion frequencies were determined by PCR analysis of 30 to 68 products per strain. Significant differences from the wild type are indicated by the single asterisk ($P < 0.02$) or double asterisks ($P < 0.005$); Fisher exact tests were used to compare all markers except X764 (*t* tests).

independently of ATP binding/hydrolysis. As in *sgs1*Δ, DSB-induced HR levels and DSB-dependent cell killing were similar in *sgs1KR*, *sgs1KA*, and the wild type (data not shown). The relatively strong crossover suppression observed in *sgs1KA* contrasts with the results of Ira et al. (26), perhaps due to differences in HR systems or SgslKA expression levels (see Discussion).

Minimal role for Sgs1 in DSB-induced direct repeat HR. To further study the role of Sgs1 in tract length and crossover control, we examined HR between *ura3* direct repeats (Fig. 4A). Direct repeat HR products include short- and long-tract gene conversions (Ura^+ Leu^+ and Ura^- Leu^+) and Leu^- deletions produced by single-strand annealing or intrachromosomal crossovers. Unequal sister chromatid exchange (USCE) produces deletions with an associated Leu^+ *ura3* triplication. DSB-induced direct repeat HR levels were similar in the wild-type and *sgs1*Δ, but unlike allelic HR, direct repeat product spectra were largely unchanged in *sgs1*Δ (Fig. 4B). In particular, *sgs1*Δ did not increase the fraction of long-tract conversions (Ura^- Leu^+). USCE comprises ~3% of Leu^+ events in wild-type cells (11). We examined 16 and 32 Leu^+ products from the wild type and *sgs1*Δ, respectively, and each set had a single USCE product (data not shown). Thus, Sgs1 does not suppress crossovers or reduce tract lengths in short (1.2 kbp) direct repeats.

The Sgs1 helicase suppresses DSB-induced chromosome loss. *sgs1*Δ shows elevated rates of spontaneous chromosome missegregation that reflect nondisjunction and, to a lesser extent, chromosome loss (69). The loss of the broken chromosome V in a diploid produces a viable aneuploid product. We found that DSB-induced chromosome loss and BIR were increased severalfold in diploid *sgs1*Δ cells (Fig. 5). Chromosome loss and BIR probably reflect problems with HR initiation, as these are the predominant outcomes in *rad51*Δ (for details see reference 35; our unpublished results). Chromosome loss was also elevated in *sgs1KR* and *sgs1KA* (Fig. 5A), but there was no significant difference in BIR between the wild type and either

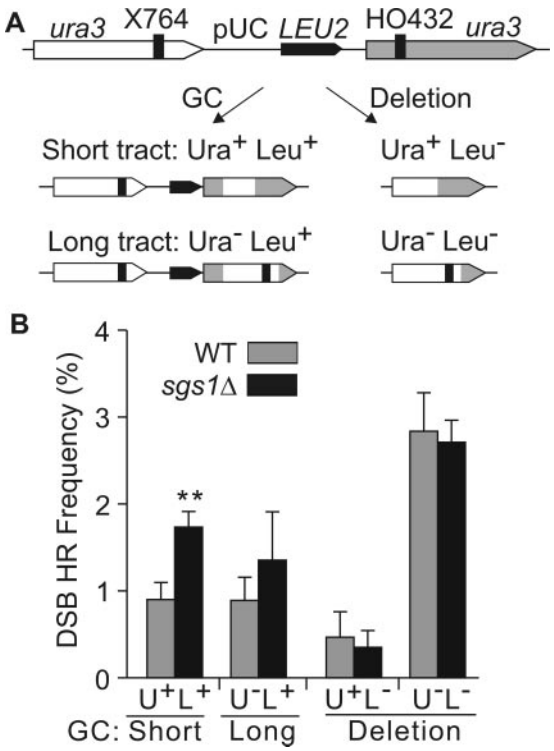

FIG. 4. Sgs1 does not suppress crossovers or tract lengths in direct repeats (A) Direct repeat HR substrate and products. *ura3* alleles, as shown in Fig. 1A, flank *LEU2* and pUC19. Two gene conversion products and two deletion products are diagrammed. (B) DSB-induced direct repeat HR frequencies, reported as described in the legend for Fig. 1. Values are averages ± SD (error bars) of four determinations. Short- and long-tract gene conversions not associated with crossovers are Leu^+. Only the Ura^+ Leu^+ category was significantly different ($P < 0.01$). Deletions (Leu^-), which can arise by single-strand annealing or crossovers, were similar in the WT and *sgs1*Δ.

helicase-defective mutant (Fig. 5B). The increased chromosome loss in the *sgs1* mutants suggests that Sgs1 has a modest role in HR initiation and that this role is helicase dependent.

The Sgs1 helicase is required for MMS resistance, suppression of *srs2*Δ lethality, and proper chromosome segregation. Haploid *sgs1*Δ cells are hypersensitive to MMS (16, 40), and haploid *sgs1KA* mutants showed similar hypersensitivities in qualitative (spot) tests (41). Quantitative analysis indicated that *sgs1KA* was more sensitive to MMS than was *sgs1*Δ (56), suggesting that Sgs1KA may act in a dominant-negative manner, but the opposite result was obtained in a subsequent study (65). These conflicting results may be due to differential expression from plasmid vectors. We performed quantitative MMS sensitivity assays and compared the wild type to *sgs1*Δ and to *sgs1KR* and *sgs1KA* expressed from the normal chromosomal locus. As shown in Fig. 6A, MMS sensitivity increased in the series WT < *sgs1*Δ < *sgs1KR* < *sgs1KA*. This pattern was apparent in haploids, and to a lesser extent, in diploids. These results indicate that the Sgs1 helicase is important for conferring resistance to MMS, and that both Sgs1KR and Sgs1KA interfere with a mechanism(s) that promotes survival during chronic MMS exposure. Given the importance of BLM in genome stabilization, ageing, and tumor suppression and reports that *BLM* heterozygous mice and humans are at

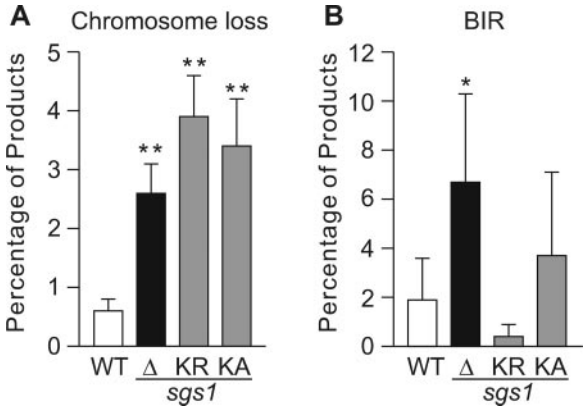

FIG. 5. Modest role for Sgs1 in HR initiation. (A) Chromosome loss was determined in >30 Ura$^-$ His$^-$ colonies per strain, and the resulting values were used to calculate percent loss values (±SD [error bars]) for each of four determinations. Double asterisks indicate *P* values of <0.0005. (B) Percent BIR was calculated by subtracting G_2 crossover and HR frequencies from total His$^-$ frequencies. Values are averages ± SD for four determinations per strain. The asterisk indicates a *P* value of 0.05.

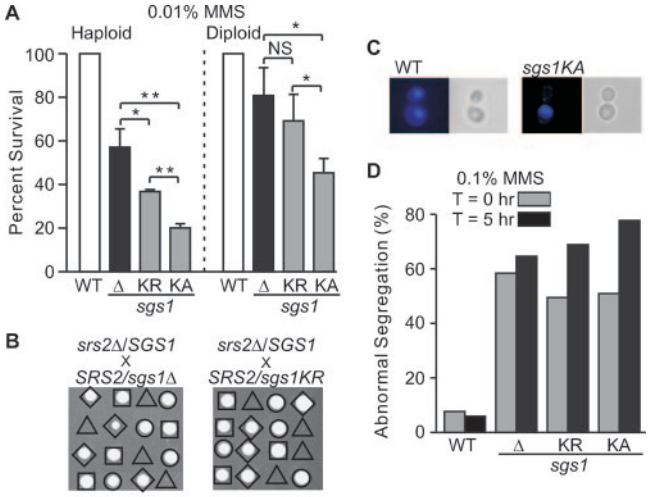

FIG. 6. Sgs1 helicase is required for MMS resistance, *srs2*Δ viability, and proper chromosome segregation. (A) Percent survival after 6 days of growth on YPD with 0.01% MMS for two determinations with the wild type and three determinations for *sgs1* mutants. Significant differences are indicated by single asterisks ($P < 0.04$) or double asterisks ($P < 0.002$). (B) Meiotic products from diploids that are heterozygous for *sgs1* and *srs2*Δ mutations. Genotypes were determined by PCR: square, WT; circle, *sgs1*; diamond, *srs2*Δ; triangle, inferred *sgs1 srs2*Δ double mutant. (C) Representative phenotypes of large-budded wild-type and *sgs1KA* mutant cells. DAPI fluorescence and light microscopic images are shown in the left and right panels, respectively, for each strain. (D) Percentage of abnormal nuclear segregation after 0 or 5 h of treatment with 0.1% MMS. For each value, we scored at least 100 large-budded cells.

increased risks for cancer (21, 22), we were interested in whether Sgs1KA or Sgs1KR exerts dominant-negative effects. We found that *SGS1*/*sgs1KR* and *SGS1*/*sgs1KA* heterozygotes showed wild-type MMS resistance (data not shown). Thus, interference with MMS resistance by Sgs1KR and Sgs1KA was apparent in only the absence of wild-type Sgs1.

*sgs1*Δ is synthetically lethal/sick with *srs2*Δ, *slx1*Δ *slx4*Δ, *slx8*Δ, *mms4*Δ, *mus81*Δ, and *rrm3*Δ; the *slx4*Δ synthetic lethality is not relieved by the expression of *sgs1KA* (42). These and other results led to the proposal that Sgs1 resolves toxic recombination intermediates at blocked or collapsed replication forks (8, 13, 15). We sporulated diploids that were heterozygous for *srs2*Δ and either *sgs1*Δ, *sgs1KA*, or *sgs1KR*, and all three *sgs1* mutants were synthetically lethal with *srs2*Δ (Fig. 6B and data not shown). *sgs1KA* was previously expressed in *srs2*Δ, but no effect was seen because the *sgs1*Δ *srs2*Δ double mutant was viable in that genetic background (42). *sgs1* suppression of *top3* slow growth (20) also points to a role for Sgs1 in replication (15). As reported by Mullen et al. (41), we found that *sgs1KA* partially suppressed *top3*Δ slow growth (data not shown). These results suggest that Sgs1 roles in replication are helicase dependent.

The reduced life span of *sgs1*Δ is due to an increased rate of normal ageing and enhanced mitotic arrest due to defective chromosome segregation (37, 69). To determine whether the Sgs1 helicase is required to suppress this defect, we examined DAPI-stained nuclei in at least 100 large-budded cells each from log-phase, wild-type *sgs1*Δ, *sgs1KR*, and *sgs1KA* cultures with or without MMS (Fig. 6C and D). Nearly all wild-type cells have a single, well-defined nucleus in each mother and daughter cell, and a 5-h treatment with 0.1% MMS had little effect. Consistent with McVey et al. (37), aberrant chromosome segregation was sharply elevated in *sgs1*Δ. Interestingly, *sgs1KR* and *sgs1KA* showed the same phenotype as *sgs1*Δ and this was exacerbated by MMS. This indicates that the Sgs1 helicase activity is critical for normal nuclear segregation in response to replication stress from endogenous and MMS-induced DNA damage.

DISCUSSION

Helicase-independent functions of Sgs1. Several helicase-independent functions of Sgs1 have been identified. For example, *sgs1KA* rescues the *sgs1*Δ sporulation defect (40, 49), increases resistance to topoisomerase inhibitors (36), and restores both MMS-induced HR (66) and hydroxyurea-induced checkpoint activation (8). Helicase-independent Rqh1 functions in DNA damage recovery and S-phase arrest have also been reported (2). Our study reveals two new helicase-independent Sgs1 functions: limiting allelic crossovers and gene conversion tract lengths.

Gene conversion tracts reflect the extent of hDNA and mismatch repair, and a number of studies have revealed connections between Sgs1 and mismatch repair proteins (44, 51). Tract lengths could increase in *sgs1*Δ if mismatch repair were compromised, as this would reduce restoration-type repair and the full hDNA tract would appear "converted" when unrepaired mismatches segregate in mitosis. However, this compromise would increase segregation of the X764 frameshift mutation and increase the frequency of Ura$^{+/-}$ colonies, but this was not observed (Fig. 1B). *Escherichia coli* RuvABC branch migrates and resolves HJs (71), but a similar complex has not been identified in eukaryotes. Instead, a significant fraction of HJs appear to be resolved by Sgs1-Top3-Rmi1 in

TABLE 2. Helicase-dependent and -independent functions for Sgs1

Suppressed function(s)	Sgs1 requirement?[a]	Reference(s)	Sgs1 helicase requirement?[b]	Reference(s)	Process
Crossovers	Yes	26; this study (Fig. 2)	No (partial?)	This study (Fig. 2)	DSB repair
Long conversion tracts	Yes	This study (Fig. 3)	No	This study (Fig. 3)	DSB repair
Chromosome loss	Modest	This study (Fig. 5A)	Modest	This study (Fig. 5A)	DSB repair
Spontaneous HR	Yes	3, 20, 44, 68	Yes	40; our unpublished results	Replication
MMS/HU[c] sensitivity	Yes	7, 16, 40, 56; this study (Fig. 6A)	Yes	This study (Fig. 6A)	Replication
Lethality in *srs2*Δ	Yes	15, 58, 64; this study (Fig. 6B)	Yes	This study (Fig. 6B)	Replication
Lethality in *slx4*Δ	Yes	18	Yes	18	Replication
Slow growth in *top3*Δ	Yes	20	Yes	41; this study (data not shown)	Replication
Nuclear abnormalities	Yes	37, 69; this study (Fig. 7D)	Yes	This study (Fig. 7D)	Chromosome segregation (replication?)

[a] Based on phenotypes observed in *sgs1*Δ.
[b] Based on phenotypes observed in *sgs1KA* or *sgs1KR*.
[c] HU, hydroxyurea.

yeast and by related complexes in higher eukaryotes. This view is supported by genetic and biochemical evidence (5, 24–26, 28, 74). The association of crossovers with long gene conversion tracts (27, 62) (Fig. 2A) and the longer tracts of *sgs1*Δ (Fig. 3) are consistent with the idea that Sgs1 reverse branch migration activity suppresses crossovers by minimizing hDNA extension. However, helicase-defective Sgs1KA and Sgs1KR suppress crossovers and minimize tract lengths like wild-type Sgs1 does (Fig. 2 and 3), indicating that Sgs1 can fulfill these roles independently of its helicase activity (discussed further below).

Ira et al. (26) reported increased crossovers in *sgs1*Δ and *sgs1KA*. We found that crossovers were only slightly elevated in *sgs1KA* (Fig. 2A). This may reflect differences in ectopic (haploid) versus allelic (diploid) HR systems, which differ in the extent of homology (2 kbp versus all of chromosome V, respectively), and in *MAT* hemizygosity versus heterozygosity, which regulates DSB repair by nonhomologous end joining and HR (46). Marked differences between allelic and ectopic HR are also evident in *srs2*Δ mutants, which show minimal cell killing during DSB-induced allelic HR but killing of >95% in the ectopic system (26). The distinct crossover results could also be due to differential Sgs1KA expression levels. Expressing *sgs1KA* and *sgs1KR* from the normal chromosomal locus gave protein levels that were indistinguishable from those of the wild type (data not shown). In the prior study, *sgs1*Δ was complemented with *SGS1* or *sgs1KA* (called *sgs1-hd*) expressed from plasmids, and although the plasmid expression levels were similar, they were not compared to endogenous Sgs1 levels. If expression of Sgs1KA from a plasmid was higher than normal, this could result in dominant-negative effects because Sgs1 function is sensitive to minor changes in protein level (13).

The suppression of crossovers and conversion tract lengths by Sgs1KA and Sgs1KR prompted us to ask whether the mutation of Lys 706 to Ala in the consensus Walker A motif (GxxxxGKT/S) was sufficient to inactivate the Sgs1 ATPase/helicase. The Walker A region in Sgs1 has a second, near-consensus motif (AvvksGKT) just seven amino acids downstream from the first motif. However, the downstream motif is unlikely to be functional because it is not conserved among known RecQ family members (60). Sgs1KA is helicase-defective in vitro (34), and *sgs1KA/KR* has several other defects in common with *sgs1*Δ, as noted below.

Helicase-dependent functions of Sgs1. By expressing *sgs1KA/KR* from the normal chromosomal locus we found that MMS resistance (Fig. 6A) and suppression of spontaneous HR (unpublished results) require a functional Sgs1 helicase, which is in agreement with prior results obtained with ectopic expression systems (40, 41). The Sgs1 helicase is also required to stabilize DNA polymerase at stalled replication forks (8, 13), suppress lethality in *slx4*Δ (42), and slow the growth rate of *top3* mutants (41) (our unpublished results). We demonstrate three new functions for the Sgs1 helicase in suppressing DSB-induced chromosome loss, abnormal nuclear segregation, and lethality in *srs2*Δ (Fig. 5A and 6B through D). In addition to the direct evidence that Sgs1 stabilizes DNA polymerase at stalled forks (8, 13), there are many other connections between DNA replication and helicase-dependent properties of Sgs1. For example, spontaneous HR is stimulated by collapsed replication forks (57) and replication forks are blocked by MMS lesions (63). The synthetic lethality of *sgs1*Δ *srs2*Δ double mutants and its suppression by defects in HR proteins are thought to reflect requirements for Sgs1 or Srs2 in processing intermediates formed by HR proteins at blocked replication forks (15, 30). The slow growth of *top3*Δ may also be due to replication problems since *top3*Δ defects are partially suppressed by defects in HR proteins (48). Finally, *slx4*Δ *sgs1*Δ synthetic lethality and the biochemical properties of the Slx1-Slx4 nuclease suggest a function for Slx4 in processing blocked replication forks (17). In light of these connections, we propose that Sgs1 helicase-dependent functions are primarily or exclusively required during DNA replication (Table 2), specifically in restarting stalled replication forks. The Sgs1 helicase is likely required to reverse regressed forks, restoring normal fork structure (Fig. 7A) (29).

The elevated chromosome loss in *sgs1* mutants could reflect the failure to resolve HR intermediates prior to mitosis, leading to chromosome missegregation. Alternatively, because chromosome loss is common in mutants that cannot initiate HR, such as *rad51*Δ (unpublished results), Sgs1 may have a

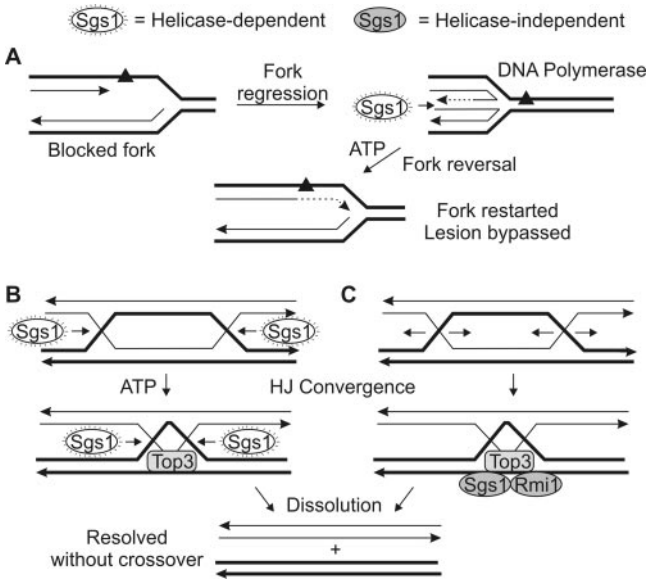

FIG. 7. Helicase-dependent and -independent roles for Sgs1. (A) Replication fork restart. A fork blocked by a DNA lesion (triangle) regresses to produce a "chicken foot" structure that allows replication past the lesion (dashed line) using the newly synthesized strand as template. Fork reversal catalyzed by the Sgs1 helicase restores the replication fork. (B) Reverse branch migration catalyzed by Sgs1 helicase converges double HJs for final resolution by Top3; modeled after Ira et al. (26). (C) Sgs1 helicase-independent reduction of crossovers and tract lengths. Double HJs migrate by random walk, and Sgs1 (plus Rmi1) stimulates Top3 activity at converged HJs. The processes depicted in panels B and C resolve intermediates without an associated crossover by HJ dissolution.

role in HR initiation. We favor the latter idea because several lines of evidence indicate that RecQ helicases promote HR initiation. For example, *E. coli* RecQ stimulates RecA-mediated strand exchange in vitro (24) and *Drosophila* BLM promotes repair synthesis (1), which could increase HR efficiency by stabilizing nascent HR intermediates. In addition, Sgs1 promotes MMS-induced HR (50), BLM and Sgs1 interact with human and yeast Rad51, respectively (72), and the deletion of *Schizosaccharomyces pombe* Rqh1 reduces HO nuclease-induced ectopic gene conversion (G. Freyer, personal communication). Although the Sgs1 role in preventing chromosome loss is helicase dependent (Fig. 5A), it is a minor role because chromosome loss in *sgs1* mutants is much less frequent than HR (Fig. 1B and 5).

The similar levels of HO-induced allelic HR in wild-type and *sgs1*Δ (Fig. 1B) contrast with the marked reduction in UV- and MMS-induced allelic HR in *sgs1*Δ (50). This could reflect any of a number of differences between HO- and UV/MMS-induced HR. For example, UV and MMS, but not HO, create more than one lesion per cell and reduce viability. The dispersed damage caused by UV and MMS also induces checkpoint arrest (67), but this is not observed with a single HO-induced DSB in diploid cells (unpublished results). In addition, Sgs1 is important for checkpoint activation, acting upstream of Rad53 (16). It is likely that UV- and MMS-induced HR results when replication forks encounter DNA lesions, whereas HO nuclease creates DSBs in a replication-independent manner. The requirement for Sgs1 in UV/MMS-induced HR may reflect specific Sgs1 roles in replication fork restart and/or checkpoint arrest; such roles would not be required for the repair of an HO-induced DSB.

A structural role for Sgs1 in crossover/tract length control. A current model suggests that the Sgs1 helicase promotes convergence of double HJs that are subsequently resolved by Top3 (Fig. 7B). However, our Sgs1KA/KR results do not support this view. Sgs1 functions in DSB repair by HR are largely helicase independent (Table 2) and likely depend on a highly conserved interaction between the Sgs1 N terminus and Top3 (23, 41, 73). Thus, Sgs1 may play a structural role in stimulating Top3 activity (23) and/or HJ recognition (5) during HR. The Sgs1 helicase is required for branch migration and/or dissolution of synthetic HJs by purified Sgs1-Top3 and BLM-Top3α in vitro (5, 24, 28, 74). This difference may reflect physical constraints imposed by the short, synthetic HJ substrates that do not allow free branch migration. Alternatively, helicase activity may be required in the absence of the Rmi1 subunit; resolving this question will require further biochemical studies with the complete complex.

If Sgs1 does not actively branch migrate HJs via its helicase activity, how can it suppress crossovers and gene conversion tract lengths? Noncrossover resolution of double HJs by dissolution might comprise separable steps of HJ branch migration and Top3-dependent decatenation stimulated by Sgs1 (and possibly Rmi1). HJ branch migration may occur passively; this follows from the suggestion by Meselson (38) that migration is energy neutral and proceeds in forward and reverse directions by a random walk, and this suggestion is supported by in vitro results (53). Thus, the Sgs1-Top3-Rmi1 complex may bind to double HJs after they converge (Fig. 7C). In this view, hDNA extension (and the risk of crossovers) increases in the absence of Sgs1 because Top3 activity is limited without the Sgs1 binding partner (23). Because Sgs1 interacts with Top3 through its N terminus, the Sgs1KR/KA defects should not affect Top3 binding, accounting for the suppression of tract lengths and crossovers by these helicase-defective proteins. A nonenzymatic role for Sgs1 in regulating tract lengths and crossovers may explain why 20 to 25% of mitotic gene conversions are associated with crossovers in wild-type cells (35, 47). These crossovers may occur when double HJs fail to converge via random walk, forcing resolution by HJ cleavage/rejoining with a 50% chance of crossing over due to HJ symmetry. If HJ cleavage/rejoining is associated with failure to converge, these events are expected to occur relatively far from the DSB, which is consistent with the greater association of crossovers with long gene conversion tracts. This model also accounts for the lack of Sgs1 regulation of crossovers and tract lengths in direct repeat HR. In these short repeats, HJ convergence by random walk would occur more frequently because branch migration is restricted by limited homology. This model predicts a greater role for Sgs1 in crossover/tract length control with increasing repeat length.

In conclusion, our results indicate that Sgs1, and BLM by extension, regulates crossovers and gene conversion tract lengths by stimulating Top3/α-dependent dissolution of double HJs. Our findings provide new insight into BLM-mediated tumor suppression. Cancer-predisposing BLM mutations occur within and outside of the helicase domain. Mutations outside the helicase domain may prevent functional interactions

with Top3α and thereby force HJ resolution toward a crossover-prone pathway, increasing the chance of large-scale loss of heterozygosity and inactivation of tumor suppressor genes. Mutations that inactivate the BLM helicase may promote tumorigenesis by preventing the restart of blocked replication forks via direct reversal of regressed forks. These structures may be shunted toward an HR-dependent restart pathway with enhanced risk of genome rearrangement.

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