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SGS1 is a multicopy suppressor of *srs2*: functional overlap between DNA helicases

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ABSTRACT

Sgs1 is a member of the RecQ family of DNA helicases, which have been implicated in genomic stability, cancer and ageing. Srs2 is another DNA helicase that shares several phenotypic features with Sgs1 and double *sgs1srs2* mutants have a severe synthetic growth phenotype. This suggests that there may be functional overlap between these two DNA helicases. Consistent with this idea, we found the *srs2*Δ mutant to have a similar genotoxin sensitivity profile and replicative lifespan to the *sgs1*Δ mutant. In order to directly test if Sgs1 and Srs2 are functionally interchangeable, the ability of high-copy *SGS1* and *SRS2* plasmids to complement the *srs2*Δ and *sgs1*Δ mutants was assessed. We report here that *SGS1* is a multicopy suppressor of the methyl methanesulphonate (MMS) and hydroxyurea sensitivity of the *srs2*Δ mutant, whereas *SRS2* overexpression had no complementing ability in the *sgs1*Δ mutant. Domains of Sgs1 directly required for processing MMS-induced DNA damage, most notably the helicase domain, are also required for complementation of the *srs2*Δ mutant. Although *SGS1* overexpression was unable to rescue the shortened mean replicative lifespan of the *srs2*Δ mutant, maximum lifespan was significantly increased by multicopy *SGS1*. We conclude that Sgs1 is able to partially compensate for the loss of Srs2.

INTRODUCTION

The ability of DNA helicases to unwind DNA enables replication, transcription, recombination and repair of DNA. The fundamental importance of DNA helicases is highlighted by a variety of human genetic disorders resulting from defective helicase function (1). Although the clinical features of these disorders differ, they all exhibit genomic instability and a predisposition to cancer. One class of DNA helicases, the RecQ family (named after its homology with the prototypical bacterial RecQ helicase), has attracted particular interest recently (2). Defects in three of the five known human RecQ family members manifest as Bloom's syndrome (*BLM*) (3), Rothmund–Thomson syndrome (*RECQ4*) (4) and Werner's syndrome (*WRN*) (5). All three disorders show genomic instability associated with a predisposition to various cancers, while Rothmund–Thomson syndrome and Werner's syndrome display features of premature ageing. At the cellular level, cells from Werner's syndrome sufferers display genomic translocations and deletions (6), and a decreased replicative lifespan *in vitro* (7, reviewed in 8). Cells from Bloom's syndrome patients similarly exhibit chromosomal rearrangements and deletions, but are characterised by a uniquely high frequency of sister chromatid exchanges (reviewed in 9).

In order to shed light on how mutations in human RecQ helicases result in genomic instability, much recent attention has been directed at RecQ orthologues in lower organisms. The genomes of *Escherichia coli*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* each contain only a single predicted RecQ helicase, thus facilitating analysis of their cellular function(s). Mutations in *E.coli recQ*, *S.cerevisiae SGS1* and *S.pombe rqh1*$^+$ all result in increased recombination (10–15), suggesting a conserved role of RecQ helicases in regulating recombination. Interestingly, the hyper-recombination phenotype of budding yeast *sgs1* mutants can be complemented by the human *WRN* and *BLM* genes (12). In addition to the hyper-recombination phenotype, *sgs1* mutants are sensitive to a range of genotoxins and display a reduced replicative lifespan (defined as the number of buds produced by a mother cell) (12,16–24). The human *BLM* gene has been shown to complement the hydroxyurea sensitivity (12) and short lifespan (22) of the *sgs1* mutant, further reinforcing the notion of a conserved function of RecQ helicases. Current thinking suggests that this function may be the prevention of inappropriate recombination at stalled replication forks arising during S phase (25).

The *S.cerevisiae SRS2* gene was discovered as a suppressor of the UV sensitivity of *rad6* and *rad18* mutants (26), and also independently in a genetic screen which isolated and characterised yeast hyper-recombination mutants (27). No obvious human homologue of Srs2 has yet been found, but the sequence of the *SRS2* gene does show homology to the bacterial UvrD and Rep helicases (28) and an *S.pombe* orthologue has been identified recently (29). One function of Srs2 is to channel the repair of DNA lesions into the *RAD6* post-replication repair pathway (30). In the absence of Srs2, DNA lesions are instead channelled into the *RAD52* homologous recombination repair pathway (31). Srs2 has also been implicated in other DNA repair processes such as non-homologous end joining (32) and single-strand annealing (33).

Srs2 shares a number of interesting features with Sgs1. Both Sgs1 and Srs2 have been demonstrated to be 3′→5′ DNA helicases

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in vitro (34,35). The *SGS1* and *SRS2* gene products are generally expressed at low abundance but are up-regulated during the S phase of the cell cycle, and both have been implicated in the intra-S checkpoint response (18,36,37). Furthermore, *sgs1* and *srs2* mutants have a hyper-recombination phenotype (13,27). Most recently, *sgs1*Δ and *srs2*Δ strains have both been shown to have a shortened replicative lifespan and a greater propensity to undergo terminal cell-cycle arrest as mitotic G_2/M intermediates (38). Deletion of both the *SGS1* and *SRS2* genes causes a severe synthetic growth defect, with low cell viability and a high incidence of G_2/M terminal cell-cycle arrest, which can be suppressed by deletion of genes involved in homologous recombination (20,38,39). This suggests that unrestrained recombination causes the poor growth of the *sgs1srs2* double mutant and is consistent with the idea that Srs2, like Sgs1, may act to prevent inappropriate recombination at stalled replication forks.

It was initially proposed that the Sgs1 and Srs2 helicases perform redundant functions in DNA replication and RNA pol I transcription (40). However, the DNA replication and RNA pol I defects reported are in fact likely to be secondary consequences of unrestrained recombination (20). Nevertheless, the original suggestion of functional overlap between these two helicases (40) remains possible. Consistent with this idea, we found the *srs2*Δ mutant to have a similar genotoxin sensitivity profile and replicative lifespan to the *sgs1*Δ mutant. Moreover, increased gene dosage of *SGS1* partially restored genotoxin resistance and increased the maximum lifespan in the *srs2*Δ mutant. This indicates that Sgs1 can partially compensate for the loss of Srs2.

Table 1. *Saccharomyces cerevisiae* strains and plasmids used in this study

STRAINS USED IN THIS STUDY	
Haploid strains	
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*
sgs1Δ	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sgs1Δ::KAN*
srs2Δ	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 srs2Δ::KAN*
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*
top3Δ	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 top3Δ::KAN*
Diploid strains	
BY4743	*his3Δ1 leu2Δ0 ura3Δ0*
sgs1Δ	*his3Δ1 leu2Δ0 ura3Δ0 sgs1Δ::KAN*
srs2Δ	*his3Δ1 leu2Δ0 ura3Δ0 srs2Δ::KAN*
yKU70Δ	*his3Δ1 leu2Δ0 ura3Δ0 yKU70Δ::KAN*
yKU80Δ	*his3Δ1 leu2Δ0 ura3Δ0 yKU80Δ::KAN*
rrm3Δ	*his3Δ1 leu2Δ0 ura3Δ0 rrm3Δ::KAN*
pif1Δ	*his3Δ1 leu2Δ0 ura3Δ0 pif1Δ ::KAN*

PLASMIDS				
Plasmid	Size (b.p.)	Plasmid type*	Selectable marker	Yeast promoter
pYES2	5900	2μ ori	*URA3* gene	*GAL1*
YCplac33	5603	*ARS1 CEN4*	*URA3* gene	none
YEp24	7769	2μ ori	*URA3* gene	none
YEplac195	5241	2μ ori	*URA3* gene	none

Yeast strains used in this study, and their genotypes, are shown. Additionally, features of plasmids used are briefly described. All plasmids contain the Amp^R selectable marker for propagation in *E.coli*.
**ARS1 CEN4* plasmids are single-copy plasmids, whereas 2μ ori plasmids are multicopy plasmids.

MATERIALS AND METHODS

Yeast strains and plasmids

Yeast strains and plasmids used in this study are described briefly in Table 1. The BY4743 strain was obtained from ATCC (Manassas, TN), and all other strains were obtained from Research Genetics (Groningen, The Netherlands). All deletion mutants were prepared by deletion module PCR using the Kan MX4 cassette (*Saccharomyces* Genome Deletion Project).

The pYES2 vector was obtained from Invitrogen (Groningen, The Netherlands). The pQE30 plasmid was obtained from Qiagen (Dorking, UK). The YCplac33 and YEplac195 vectors were gifts from Dr Alan Boyd (University of Edinburgh, Edinburgh, UK). The pNF3000 plasmid, which carries the *RAD3* gene controlled by its endogenous promoter in the YEp24 vector, was generously provided by Drs Paula Fischaber and Errol Friedberg (University of Texas Southwestern Medical Center, Dallas, TX).

Plasmid construction

The YCplac33-*SRS2* and YEplac195-*SRS2* plasmids were constructed as follows. The 3525 bp *SRS2* open reading frame (ORF) plus 610 bp upstream and 132 bp downstream was amplified from yeast genomic DNA (Promega, Southampton, UK) using the following primers: CATGAACCTGGATCCATATATAGAAATCGG (sense, *Bam*HI site underlined) and CATTAAGGGTACCTAACACACCATCCACATTTC (antisense, *Kpn*I site underlined). The PCR product was digested with *Bam*HI and *Kpn*I and ligated into *Bam*HI/*Kpn*I-digested YCplac33 and YEplac195 vectors. The pYES2-*SRS2* plasmid was constructed by amplifying the 3.525 kb *SRS2* ORF from YEplac195-*SRS2* using the primers GGGAGGATCCATGTCGTCGAACAATGATCTTTG (sense, *Bam*HI site underlined) and GAAACTCGAGCTAATCGATGACTATGATTTCAC (antisense, *Xho*I site underlined). The PCR product was digested with *Bam*HI and *Xho*I and ligated into *Bam*HI/*Xho*I-digested pYES2 vector.

The YEplac195-*SGS1* and YEplac195-*SGS1* $K^{706}\rightarrow A$ constructs were made by subcloning the 4.8 kb *Sal*I fragment containing the *SGS1* gene from YCplac33-*SGS1* and YCplac33-*SGS1* $K^{706}\rightarrow A$ (23) into *Sal*I-digested YEplac195. The pYES2-*SGS1* plasmid was constructed by amplifying the 4.344 kb *SGS1* ORF from yeast genomic DNA using the following primers: GTACACACAAGGGATCCATGGTGACGAAGC (sense, *Bam*HI site underlined) and TTTCCTCGAGTCACTTTCTTCCTCTGTAGT (antisense, *Xho*I site underlined). The PCR product was digested with *Bam*HI and *Xho*I and ligated into *Bam*HI/*Xho*I-digested pYES2 vector.

The pQE30-*SGS1*(1-400) plasmid was constructed as follows. The 1200 bp fragment of *SGS1* was amplified from YCplac33-*SGS1* using the following primers: GTACACACAAGGGATCCATGGTGACGAAGC (sense, *Bam*HI site underlined) and TTCTTCTGGTACCACCTACGGATAATCGTC (antisense, *Kpn*I site underlined). The PCR product was digested with *Bam*HI and *Kpn*I and ligated into *Bam*HI/*Kpn*I-digested pQE30 plasmid.

Construction of *SGS1* mutant libraries

Initially, the YEplac195-*SGS1* plasmid was modified to remove a *Not*I restriction site carried over from the original cloning vector. Transposon-based mutagenesis was then performed on this modified plasmid using the E::Z™ In-frame linker insertion kit (Cambio, Cambridge, UK). This utilises the Tn5 *in vitro* transposition system (41). Mutagenesis was performed *in vitro* by incubating 0.03 pmol of EZ::TN<*Not*I/KAN-3> transposon and plasmid DNA with 1 µl of Tn5 transposase enzyme at 37°C for 2 h. The reaction was then stopped and the reaction mixture transformed into XL1 Blue *E.coli* (Stratagene, Amsterdam, The Netherlands). All resultant Kan^+ Amp^+ colonies were pooled and grown up prior to plasmid DNA isolation. The Kan^R gene was spliced out by digesting the midiprep with *Not*I, leaving a 57-bp sequence at the site of transposon insertion. This sequence codes for a 19-amino acid read-through insertion, which maintains the reading frame and so avoids creation of truncation mutants. The *Not*I-cut plasmid was gel purified, re-ligated *in vitro* and re-transformed into XL1 Blue *E.coli*. The resulting Amp^+ colonies were used to construct both the random mutational library and the methyl methanesulphonate (MMS) loss-of-function library.

For the random mutational library, 190 Amp^+ colonies were selected at random and grown up individually. Of these, PCR analysis identified 80 colonies that contained plasmids harbouring the 57-bp insertion within the *SGS1* gene. These colonies were individually grown up prior to isolation of their plasmid DNA. The position of transposon insertion was determined by digesting plasmid minipreps with *Sal*I and *Not*I.

For the MMS loss-of-function library, all original Amp^+ colonies harbouring transposon-mutagenised YEplac195-*SGS1* were pooled and grown up. Their DNA was then isolated and transformed into the *sgs1*Δ mutant strain. Ura^+ transformants (500) were then picked and patched individually onto 10 × 10 grids on SD-URA plates. Each plate also contained positive (*sgs1*Δ-YEplac195-*SGS1*) and negative (*sgs1*Δ-YEplac195) controls for comparison. Plates were incubated overnight at 30°C, and then replica plated onto plates containing 0.023% MMS and left for 2 days. Loss-of-function mutations (i.e. plasmids no longer able to rescue MMS sensitivity in the *sgs1*Δ strain) were identified on replica plates, and the corresponding colonies picked from the master plate. These were grown up, pooled and their DNA recovered and transformed into XL1 Blue *E.coli*. Forty-two Amp^+ colonies were selected for further analysis of transposon insertion by restriction digest analysis. The remaining colonies were pooled, and their DNA isolated by midiprep.

Drug sensitivity plates

This was performed as described previously for *sgs1*Δ, using identical drug concentrations (23). For quantitation of strains grown on solid media supplemented with MMS, yeast strains were grown up in minimal media for 2–3 days at 30°C, diluted and then spread on solid media plates containing increasing doses of MMS. After 3 days, the percent viability of each strain was expressed as number of colonies obtained relative to that on the control (no MMS) plate. For quantitation of MMS sensitivity of strains in liquid media, yeast strains were grown to an OD_{600} of 0.5–0.6 before addition of 0.01–0.12% MMS for 60 min. Cells were washed in 10% sodium thiosulfate, diluted and spread onto solid media plates. After 3 days, the percent viability of each strain was expressed as number of colonies obtained relative to that obtained for the control samples.

Detection of Sgs1 and Srs2 by western blotting

Yeast extracts were prepared by bead-beating in 8 M-urea-containing 2D-gel extraction buffer (Bio-Rad, Hemel Hempstead, UK) and run on SDS–polyacrylamide gels prior to transfer onto nitrocellulose. Recombinant his-tagged Sgs1(1-400) protein was purified from *E.coli* transformed with the pQE30-*SGS1*(1-400) plasmid using Ni^{2+}-NTA-agarose (Qiagen). The purified protein was then used for antibody production in rabbits by following a published method (42). The resulting Sgs1p 857 antiserum was then purified using protein G–Sepharose to yield the IgG fraction used for western blotting. The Srs2p (yA-19) and Sir2p (yN-19) goat polyclonal antibodies were obtained from Autogen Bioclear (Mile Elm, UK). All primary antibodies were routinely used at dilutions of 1:500. Immunoreactive bands were detected with peroxidase-conjugated secondary antisera and visualised using enhanced chemiluminescence.

Lifespan analysis

This was performed as described previously (23). Briefly, strains were grown at 30°C until they reached an OD_{600} of 0.6–1.0. One microlitre of culture was streaked onto plates and left at 30°C for 1–2 h. After this time, cell doublets were moved to uninhabited regions of the plate. When these budded again, (newly formed) virgin yeast cells were removed by micromanipulation to a new location. All future buds produced by these daughter cells were micromanipulated away, and catalogued. The plates were incubated at 30°C during working hours, and moved to 4°C overnight. Lifespan was defined as number of daughter cells removed from the mother cell. All lifespans were observed a minimum of twice.

Yeast transformation and DNA extraction

This was performed as described previously (23).

RESULTS

Deletion of the *SGS1* gene in both haploid and diploid strains has been reported to result in a replicative lifespan of ~40% that of isogenic wild-type strains (22–24). To determine whether deletion of *SRS2* has a similar effect, lifespan analysis was performed on *srs2*Δ and BY4743 (wild-type) isogenic diploid strains transformed with the empty YCplac33 vector (Fig. 1A). Both strains were transformed with YCplac33 to allow direct comparison with previous findings (23). The mean lifespan of the BY4743-YCplac33 strain was 16.0 ± 1.0 (n = 76), compared with 7.2 ± 0.9 (n = 38) for the *srs2*Δ-YCplac33 strain. The maximum lifespans recorded were 44 and 23, respectively. The short lifespan of the *srs2*Δ mutant observed is comparable with that of the isogenic diploid *sgs1*Δ-YCplac33 strain, which has a mean lifespan of 7.4 ± 0.8 (n = 32) and maximum of 16 (23). A similar magnitude of shortened replicative lifespan has recently been reported for the haploid *sgs1*Δ and *srs2*Δ strains (38). Therefore, deletion of *SGS1* or *SRS2* reduces the replicative lifespan to a similar extent in both haploid and diploid backgrounds.

We have previously characterised the sensitivity of the *sgs1*Δ mutant to MMS (DNA methylating agent), 4-NQO

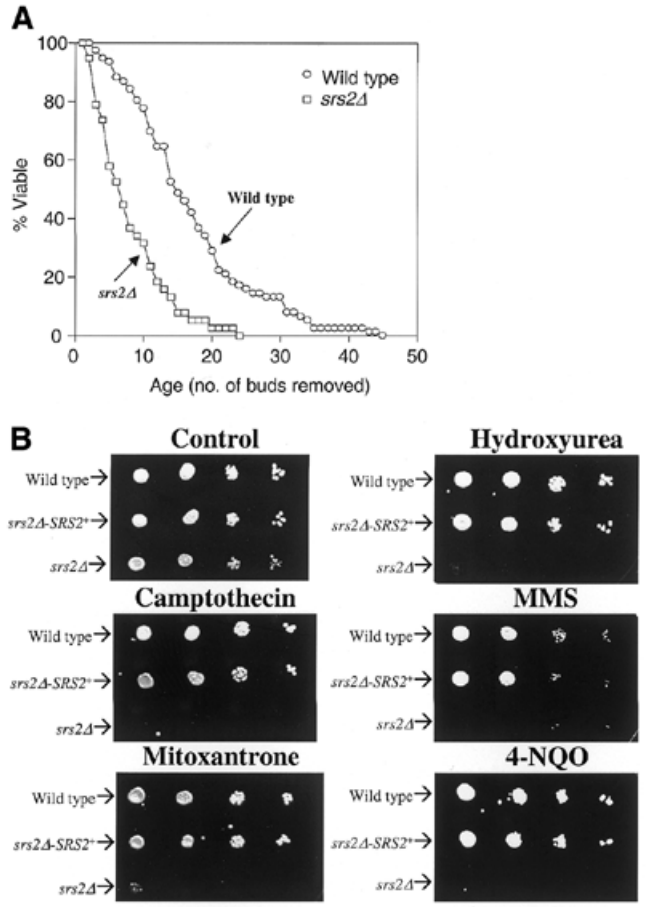

Figure 1. The diploid *srs2*Δ mutant has a shortened replicative lifespan and is sensitive to genotoxins. (**A**) Lifespan analysis. The *srs2*Δ and isogenic wild-type BY4743 diploid strains were transformed with YCplac33 vector and the replicative lifespan determined manually by micromanipulation. Individual cells were followed until they ceased dividing, and replicative age was defined as the number of buds removed from a mother cell. The survivorship curve shows the percentage of the population of yeast cells that is viable plotted against replicative age. Average lifespan of the strains (expressed as mean number of buds removed ± SEM) was 16 ± 1.0 (n = 76) for the BY4743-YCplac33 strain and 7.2 ± 0.9 for the *srs2*Δ-YCplac33 strain (n = 38). Maximum lifespans recorded were 44 and 23, respectively. (**B**) Genotoxin sensitivity. Strains were grown up, diluted and serially spotted out onto solid media plates containing various drugs. Plates were left for 3–4 days at 30°C. Cell spots (from left to right) correspond to serial 1 in 10 dilutions of cells (starting, furthest left, with OD_{600} = 0.33). The diploid strains compared were *srs2*Δ-YCplac33 (*srs2*Δ), *srs2*Δ-YCplac33-*SRS2* (*srs2*Δ-*SRS2*$^+$) and the wild-type control, BY4743-YCplac33 (BY4743). Drug doses used were as follows: 50 mM hydroxyurea, 0.33 mM camptothecin, 0.0066% MMS, 1 mM mitoxantrone and 0.5 μM 4-NQO.

(causes bulky-base adducts and oxidative damage), hydroxyurea (DNA synthesis inhibitor), camptothecin (Type I topoisomerase inhibitor) and mitoxantrone (Type II topoisomerase inhibitor) (23). To assess the sensitivity of the *srs2*Δ mutant to these drugs, the diploid *srs2*Δ mutant was transformed with YCplac33 (empty vector) and YCplac33-*SRS2* and compared with the BY4743 isogenic wild-type strain transformed with YCplac33. Various dilutions of *srs2*Δ-YCplac33, BY4743-YCplac33 and *srs2*Δ-YCplac33-*SRS2* strains were grown on plates containing the above drugs (Fig. 1B). Similar to the *sgs1*Δ mutant, the *srs2*Δ-YCplac33 strain showed sensitivity to all DNA damaging agents tested. Transformed *srs2*Δ cells containing the YCplac33-*SRS2* plasmid showed comparable drug sensitivity to the BY4743 wild-type strain under these conditions. The observation that plasmid-borne *SRS2* can restore genotoxin resistance to isogenic wild-type levels thus confirms that deletion of the *SRS2* gene is the sole defect in the *srs2*Δ mutant.

To assess the specificity of the drug sensitive phenotype of the *sgs1*Δ and *srs2*Δ strains, the MMS sensitivity of other yeast mutants lacking DNA helicases was assessed. The *pif1*Δ, *rrm3*Δ, *yKU70*Δ and *yKU80*Δ mutants were tested. Pif1 and Rrm3 are DNA helicases involved in regulating telomeric and mitochondrial DNA (reviewed in 43), and are also involved in the replication fork progression through the rDNA (44). The *yKU70* and *yKU80* gene products are involved in the repair of DNA double strand breaks (reviewed in 45) and physical and genetic interactions between RecQ helicases and Ku proteins have been demonstrated (46,47). It was found that the *pif1*Δ mutant showed a slight sensitivity to MMS, as compared with the isogenic wild-type strain. The *rrm3*Δ, *yKU70*Δ and *yKU80*Δ strains were not sensitive to MMS (results not shown). Therefore, MMS sensitivity appears to be a relatively specific phenotype for loss-of-function of either the Sgs1 or Srs2 helicases.

Based on the fact that either single mutant is viable, but the double mutant has a severe growth defect, it has been proposed that the Sgs1 and Srs2 helicases may be functionally redundant (40). We therefore reasoned that overexpression of either of these DNA helicases might compensate for loss of the other. To initially confirm overexpression, Sgs1 and Srs2 levels *in vivo* were assessed by western blotting. Protein was extracted from *sgs1*Δ-pYES2, *sgs1*Δ-pYES2-*SGS1*, *srs2*Δ-pYES2 and *srs2*Δ-pYES2-*SRS2* cultures induced with galactose. Extracted protein samples were separated by SDS–PAGE, western blotted and probed for either Sgs1 or Srs2. An ~160 kDa band corresponding to Sgs1 was present in the *sgs1*Δ-pYES2-*SGS1*, but not *sgs1*Δ-pYES2, protein extract (Fig. 2A). Similarly, an ~150 kDa band corresponding to Srs2 was present in the *srs2*Δ-pYES2-*SRS2*, but not *srs2*Δ-pYES2, protein extract (Fig. 2A). For a control, all samples were probed for Sir2p, which was equally present in all protein extracts.

Although expression of *SGS1* or *SRS2* from the galactose-inducible pYES2 vector resulted in detectable levels of Sgs1 or Srs2 protein, it was not possible to detect Sgs1 by western blotting when *SGS1* was expressed under its own promoter from the YEplac195 vector. We therefore attempted to genetically verify the overexpression of Sgs1 from this plasmid. Sgs1 helicase activity has been postulated to produce a substrate that is deleterious unless resolved by Top3 (11,48). Sgs1 overexpression should therefore be more harmful in *top3*Δ mutants than in *TOP3* strains. To test this, *top3*Δ mutant and isogenic wild-type control strains were transformed with both low-copy (YCplac33) and high-copy (YEplac195) *SGS1* plasmids (Fig. 2B). Both strains gave viable Ura$^+$ transformants when transformed with the YCplac33, YCplac33-*SGS1*, YEplac195 and YEplac195-*SGS1* $K^{706}\rightarrow A$ plasmids. However, transformation of both strains with YEplac195-*SGS1* gave viable Ura$^+$ transformants in the wild-type strain, but very few viable colonies with the *top3*Δ mutant. Therefore, expression of Sgs1 from the multicopy YEplac195 vector, but not the low-copy YCplac33 plasmid, was detrimental to the *top3*Δ mutant. This indicates that increased gene dosage of Sgs1 does indeed result in

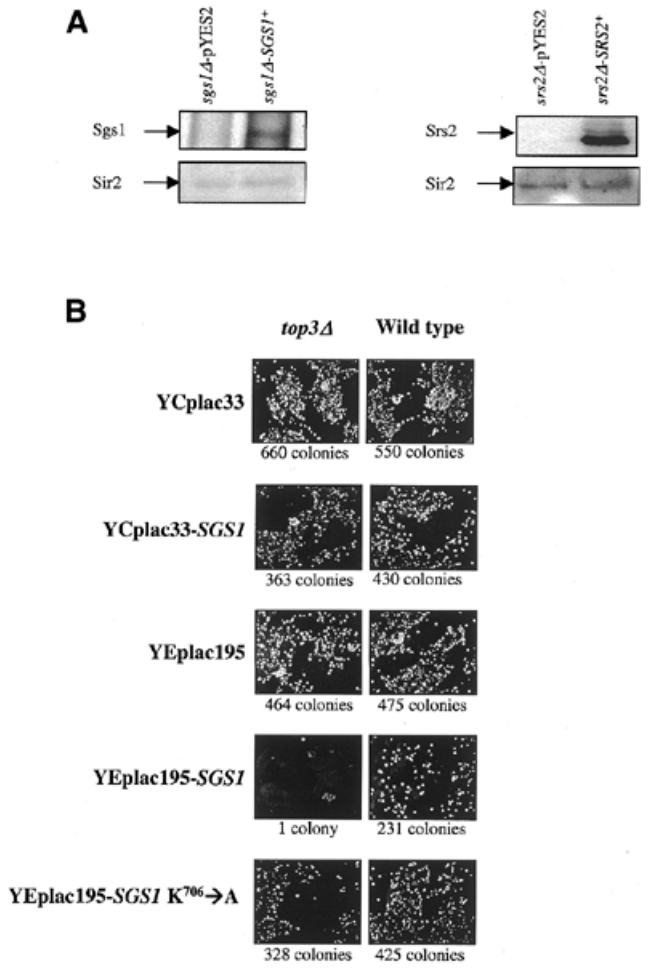

Figure 2. Confirming overexpression of the Sgs1 and Srs2 helicases *in vivo*. (**A**) The ability to detect Sgs1 and Srs2 *in vivo* was assessed by western blotting. Protein was extracted from the *sgs1*Δ-pYES2, *sgs1*Δ-pYES2-*SGS1*, *srs2*Δ-pYES2 and *srs2*Δ-pYES2-*SRS2* strains incubated in galactose-containing media to induce expression. Yeast proteins were extracted, separated by SDS–PAGE and probed for Sgs1 or Srs2. For control, the samples were probed for Sir2. (**B**) Transformation of the *MAT***a** *top3*Δ mutant and isogenic wild-type control BY4742 strains with both low-copy (YCplac33) and high-copy (YEplac195) *SGS1* plasmids was compared. Both strains were transformed with equal amounts of YCplac33, YCplac33-*SGS1*, YEplac195, YEplac195-*SGS1* and YEplac195-*SGS1* $K^{706}\rightarrow A$ plasmids. Equivalent amounts of each transformation mixture were spread on SD-URA solid media plates and incubated at 30°C. After 3 days, plates were assessed for numbers of Ura^+ transformants.

increased Sgs1 protein levels. Furthermore, the deleterious effects of higher Sgs1 levels in the *top3*Δ mutant required the helicase activity of Sgs1, consistent with the notion that the helicase activity of Sgs1 creates a substrate that Top3 resolves (11,48).

To assess if Sgs1 and Srs2 are functionally interchangeable, the ability of high-copy plasmids carrying *SGS1* and *SRS2* to rescue MMS sensitivity in the *sgs1*Δ and *srs2*Δ strains was assessed (Table 2). To control for non-specific effects of helicase overexpression, the high-copy pNF3000 plasmid carrying *RAD3* (a 5′→3′ DNA helicase involved in excision repair) (49) was also compared. As a further specificity control, the effects of these plasmids on the (slight) MMS sensitivity of the *pif1*Δ mutant were also investigated. High-copy plasmids encoding

Table 2. Ability of various plasmids to rescue the MMS sensitivity of the diploid *sgs1*Δ, *srs2*Δ or *pif1*Δ strains

	ABILITY TO RESCUE MMS SENSITIVITY		
PLASMID	*sgs1Δ*	*srs2 Δ*	*pif1Δ*
YEplac195	-	-	-
YEplac195-*SGS1*	+	+	-
YEplac195-*SGS1* $K^{706}\rightarrow A$	-	-	-
YEplac195-*SRS2*	-	+	-
pYES2	-	-	N.D.
pYES2-*SGS1*	+	+	N.D.
pYES2-*SRS2**	-	+	N.D.
pNF3000 (YEp24-*RAD3*)	-	-	-

All vectors used are 2μ-based high-copy plasmids. The YEplac195 and YEp24 plasmids possess the genes of interest under the control of their own endogenous promoter. The pYES2-based vectors contain the gene of interest under the control of the inducible *GAL1* promoter. The pNF3000 plasmid contains the *RAD3* gene under control of its own endogenous promoter. Ability to rescue MMS sensitivity is defined as clearly increased colony growth relative to an empty vector control. +, rescue; –, no rescue; N.D., not determined.
*Plasmids grown in raffinose medium, which allows 'leaky' expression from the *GAL1* promoter. Strains containing the pYES2-*SRS2* plasmid failed to grow in galactose-containing medium.

SRS2 (both YEplac195-*SRS2* and pYES2-*SRS2*) were able to complement the MMS sensitivity of the *srs2*Δ mutant, but had no obvious beneficial effects in either the *sgs1*Δ or *pif1*Δ mutants. Rather, Srs2 overexpression was found to have a dominant negative effect on growth of *sgs1*Δ, *pif1*Δ and wild-type strains. High-copy vectors expressing *SGS1* (YEplac195-*SGS1* and pYES2-*SGS1*) were able to complement the MMS sensitivity of both the *sgs1*Δ and *srs2*Δ mutant, but not the *pif1*Δ mutant. Sgs1 helicase activity was essential for complementation as YEplac195-*SGS1* $K^{706}\rightarrow A$ (expressing a helicase-defective allele which has some complementing activity in the *sgs1*Δ mutant) (23,50) was not able to suppress the MMS sensitivity of the *sgs1*Δ or *srs2*Δ mutant. The pNF3000 plasmid was unable to rescue the MMS sensitivity of all three mutants tested, although this plasmid has been demonstrated previously to complement the UV sensitivity of a *rad3-1* strain (49).

The ability of *SGS1* to rescue the sensitivity of the *srs2*Δ mutant to MMS and hydroxyurea is shown in Figure 3A. This demonstrates that the ability of *SGS1* to act as a multicopy suppressor of *srs2*Δ is not uniquely specific for sensitivity to DNA damaging agents, but is also seen for inhibitors of DNA replication. To quantify the *SGS1* complementation of *srs2*Δ MMS sensitivity, *srs2*Δ-YEplac195-*SGS1*, *srs2*Δ-YEplac195 and BY4743-YEplac195 diploid strains were grown up, diluted and then spread onto plates containing increasing doses of MMS. After 3 days, the percent viability of each strain was expressed as number of colonies obtained relative to that on the control (no MMS) plate. It can be seen that at the 0.003% dose, where complete killing of *srs2*Δ-YEplac195 cells occurred, the YEplac195-*SGS1* construct restored MMS resistance to ~60% of that seen in the isogenic wild-type (Fig. 3B). A similar effect

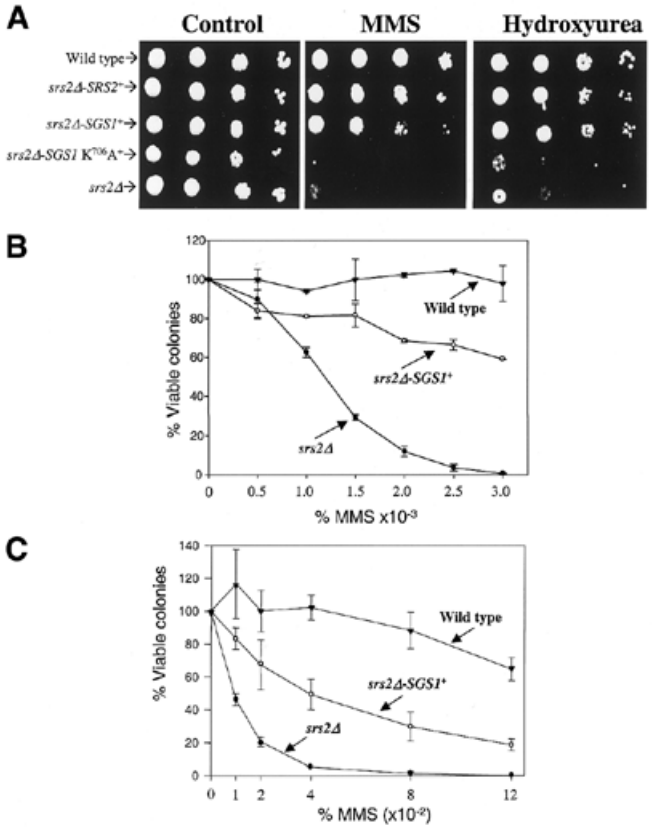

Figure 3. *SGS1* is a multicopy suppressor of the drug sensitivity of the *srs2*Δ mutant. (**A**) The diploid *srs2*Δ mutant was transformed with a range of constructs subcloned into the high-copy vector, YEplac195. Strains were grown up in minimal media, then diluted and serially spotted out onto control (no drug) plates and plates containing either 0.0062% MMS or 50 mM hydroxyurea. Plates were left at 30°C for 3 days. Cell spots (from left to right) correspond to serial 1 in 10 dilutions of cells. (**B**) To quantify the *srs2*Δ-complementing ability of *SGS1*, strains were grown up as above and then spread onto plates containing increasing doses of MMS. The number of colonies visible after 3 days was then counted on each plate. For each strain, percent viability was expressed as a percentage of viable colonies obtained on the control (no MMS) plates. The diploid strains compared were *srs2*Δ-YEplac195 (*srs2*Δ), *srs2*Δ-YEplac195-*SGS1* (*srs2*Δ-*SGS1*$^+$) and the isogenic wild-type control, BY4743-YEplac195 (Wild type). Data presented are pooled from two independent experiments and are expressed as mean ± range. (**C**) To quantify the *srs2*Δ-complementing activity of *SGS1* in liquid media, strains were grown up before addition of MMS for 60 min. MMS was then inactivated, washed off and yeast spread on solid media plates. The number of colonies visible after 3 days was then counted on each plate. For each strain, percent viability was expressed as a percentage of viable colonies obtained for the control (no MMS) samples. The diploid strains compared were *srs2*Δ-YEplac195 (*srs2*Δ), *srs2*Δ-YEplac195-*SGS1* (*srs2*Δ-*SGS1*$^+$) and the isogenic wild-type control, BY4743-YEplac195 (Wild type). Data presented are pooled from two independent experiments and are expressed as mean ± range.

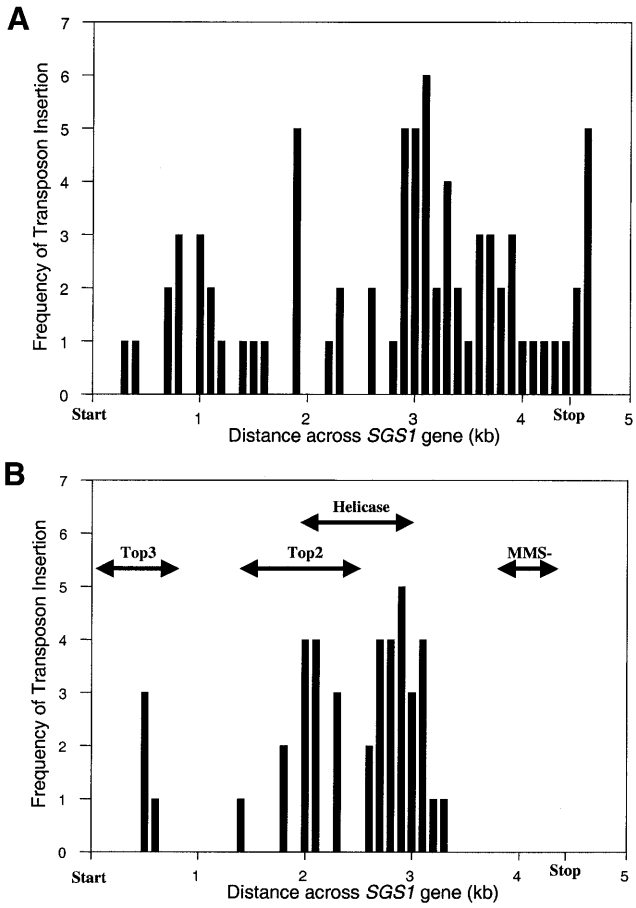

Figure 4. Transposon-scanning mutagenesis of *SGS1*. (**A**) Bar chart showing frequency and location of in-frame transposon insertions in a non-selected library. The approximate location of transposon insertion was determined for 75 randomly chosen alleles by analysis of restriction endonuclease digests of mutagenised *SGS1* plasmids. (**B**) Bar chart showing frequency and location of in-frame transposon insertions that abolish MMS complementation. Alleles of *SGS1* (100) were selected which failed to rescue the MMS sensitivity of the *sgs1*Δ mutant. Forty-two of these alleles had their location of transposon insertion determined by analytical restriction digest mapping. The approximate positions of regions required for interaction with Top2 and Top3, the helicase domain and a region dispensable for MMS resistance (*MMS*–) are indicated.

was observed when cells were exposed to MMS for 1 h before removal and inactivation of the drug (Fig. 3C). Under these conditions, overexpression of *SGS1* restored viability to 30–40% of wild-type levels at doses where complete killing of control *srs2*Δ-YEplac195 cells occurred.

Mutational analysis suggests that Sgs1 is a multifunctional protein, as mutant alleles of *sgs1* have selective complementing ability in different phenotypic assays (17,21,23,51). We therefore set out to determine whether the domains of Sgs1 required for rescue of MMS sensitivity in the *sgs1*Δ mutant differ from those required for complementation of the *srs2*Δ mutant. In order to do this, we used *in vitro* transposon-scanning mutagenesis on the YEplac195-*SGS1* plasmid to create two distinct libraries of *sgs1* mutants containing in-frame 19-amino acid insertions (see Materials and Methods). The unselected mutational library was generated by individually growing up randomly chosen bacterial colonies and PCR screening for the presence of plasmids harbouring the 57-bp insertion within the *SGS1* gene. Figure 4A shows the frequency and approximate location of in-frame transposon insertions in *SGS1* in this library, which comprises 75 alleles. It can be seen that there is coverage of insertion events throughout the entire *SGS1* gene with no obvious hotspots, suggesting that transposon insertion is basically random. To construct a library of *sgs1* MMS loss-of-function plasmids (i.e. plasmids no longer able to rescue MMS sensitivity in the *sgs1*Δ strain), *sgs1*Δ cells were transformed with the entire original pool of transposon-mutagenised

YEplac195-*SGS1*. Of the resulting Ura$^+$ transformants, 500 were picked and grown up individually on 10 × 10 grids before replica plating onto MMS plates. One hundred colonies failed to grow on MMS plates; these were recovered from the master plate, and their pooled plasmid DNA isolated to create the MMS loss-of-function library. Restriction analysis of 42 alleles chosen at random from this library revealed a more restricted distribution of insertion events than in the unselected library (compare Fig. 4A and B). Most notably, there was a complete absence of insertions in the C-terminal 250 amino acids of Sgs1, a domain known to be dispensable for MMS resistance (17,51) (Fig. 4B).

To determine whether the same functional domains of *SGS1* are also required for complementation of *srs2*Δ, the *srs2*Δ mutant was transformed with the MMS loss-of-function *SGS1* library (more than 500 Ura$^+$ transformants obtained). For a control, the *srs2*Δ mutant was also transformed with the unselected library of 75 transposon-mutagenised *SGS1* plasmids characterised in Figure 4A (more than 200 Ura$^+$ transformants obtained). Colonies were resuspended, diluted and spread on control (no MMS) and 0.0035% MMS plates (a dose at which the *srs2*Δ mutant fails to grow). For the unselected *SGS1* plasmid library, 156 colonies were obtained in total, compared with 378 colonies obtained on the control (no MMS) plates. This verifies that insertion of the 57-bp linker sequence in the *SGS1* gene is not detrimental *per se*. In contrast, for the MMS loss-of-function *SGS1* plasmid library, none of the MMS loss-of-function plasmids (in the *sgs1*Δ background) could rescue MMS sensitivity in the *srs2*Δ mutant. Therefore, we conclude that the same domains of *SGS1* are required to restore MMS resistance to both *sgs1*Δ and *srs2*Δ strains, suggesting that a common molecular mechanism of action underlies complementation in both mutants.

It has been reported that sensitivity to MMS is enhanced in diploid *srs2*Δ strains relative to haploids (28). This ploidy-specific phenotype has been interpreted as additional lethal recombination events occurring between homologous chromosomes as well as sister chromatids. We therefore set out to determine whether the ability of *SGS1* to complement MMS sensitivity in the *srs2*Δ mutant is due to suppression of recombination between homologous chromosomes. Haploid *sgs1*Δ and *srs2*Δ strains were used for these studies and compared with the isogenic wild-type BY4741 strain. To directly compare any ploidy-specific phenotypes, both haploid and diploid BY4741, *sgs1*Δ and *srs2*Δ mutant strains were grown on plates ± MMS (Fig. 5A). As reported previously (28), the diploid *srs2*Δ strain was more MMS sensitive than the haploid *srs2*Δ strain. The observed MMS sensitivity of strains (in order of most MMS sensitive) was as follows: *srs2*Δ diploid > *sgs1*Δ haploid = *sgs1*Δ diploid > *srs2*Δ haploid > BY4741 haploid = BY4741 diploid.

To test for any ploidy-specific effects of Sgs1 and Srs2 overexpression, haploid strains transformed with YEplac195, YEplac195-*SGS1*, YEplac195-*SGS1* K^{706}→A or YEplac195-*SRS2* were grown on plates ± MMS. Both the haploid *sgs1*Δ-YEplac195 and *srs2*Δ-YEplac195 strains were sensitive to 0.01% MMS, whereas BY4741-YEplac195 was unaffected at these doses (Fig. 5B). The ability of YEplac195-*SGS1* (and not YEplac195-*SGS1* K^{706}→A) to suppress MMS sensitivity was reproducible in both the *sgs1*Δ and *srs2*Δ haploid strains. Similarly, YEplac195-*SRS2* could rescue the MMS-sensitive

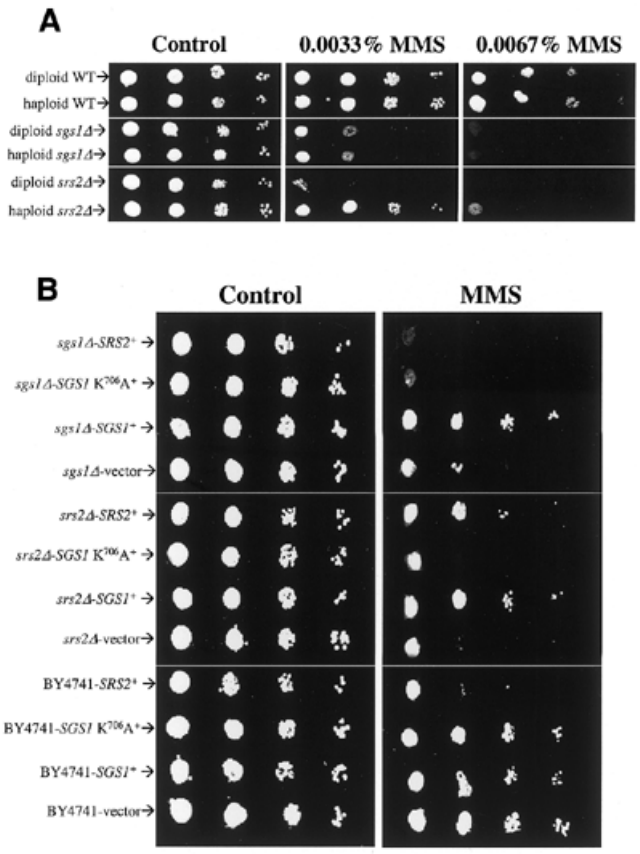

Figure 5. Complementation of *srs2*Δ MMS sensitivity by *SGS1* is ploidy independent. (**A**) MMS sensitivity of haploid and diploid wild-type, *sgs1*Δ and *srs2*Δ strains. Strains transformed with the empty YEplac195 vector were grown up, diluted and spotted out onto plates ± MMS. Plates were left at 30°C for 3 days. Cell spots (from left to right) correspond to serial 1 in 10 dilutions of cells. (**B**) MMS sensitivity of *MAT***a** haploid wild-type, *sgs1*Δ and *srs2*Δ strains transformed with high-copy *SGS1* and *SRS2* plasmids. Each strain was transformed with YEplac195 (vector), YEplac195-*SRS2* (*SRS2*$^+$), YEplac195-*SGS1* (*SGS1*$^+$) and YEplac195-*SGS1* K^{706}→A (*SGS1* K^{706}→A$^+$). Strains were grown up in minimal media, then diluted and serially spotted out onto control (no MMS) plates and plates containing 0.01% MMS. Plates were left at 30°C for 3 days. Cell spots (from left to right) correspond to serial 1 in 10 dilutions of cells.

phenotype of the haploid *srs2*Δ mutant, but again no beneficial effects in the *sgs1*Δ haploid were evident. This demonstrates that the ability of *SGS1* to function as a multicopy suppressor of the *srs2*Δ mutant is not a ploidy-specific effect.

In addition to the MMS-sensitive phenotype, the ability of the YEplac195-*SGS1* plasmid to rescue the shortened replicative lifespan of the *srs2*Δ mutant was investigated. Lifespan analysis was performed on the *srs2*Δ-YEplac195 and *srs2*Δ-YEplac195-*SGS1* strains (Fig. 6). Average lifespans of the strains [expressed as mean number of buds removed ± standard error of the mean (SEM)] were 11.1 ± 1.2 (n = 37) for *srs2*Δ-YEplac195 and 13.3 ± 1.4 (n = 38) for *srs2*Δ-YEplac195-*SGS1*. The maximum lifespans recorded were 24 and 36, respectively. Although a Student's t-test revealed there to be no significant difference between the mean lifespans, a χ^2 test on the 10% longest-lived subpopulations (52) revealed that Sgs1 overexpression caused a statistically significant increase in *srs2*Δ lifespan ($P < 0.02$).

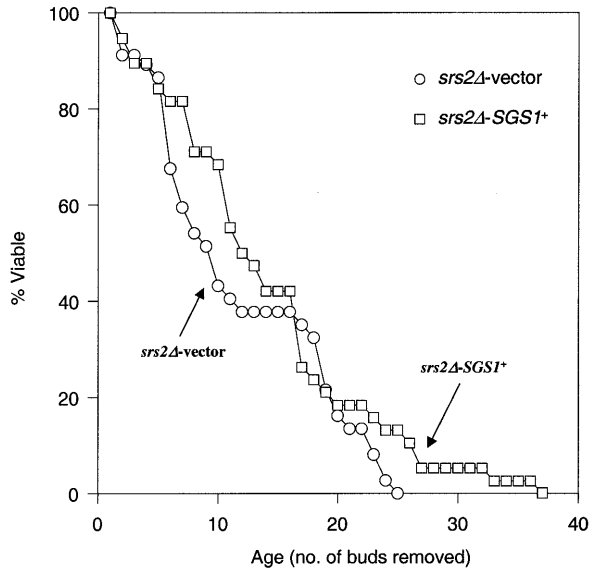

Figure 6. Effect of multicopy *SGS1* on the short lifespan of the *srs2*Δ mutant. The diploid *srs2*Δ mutant strain was transformed with YEplac195 and YEplac195-*SGS1* and the replicative lifespan determined manually by micromanipulation. Individual cells were followed until they ceased dividing, and replicative age was defined as the number of buds removed from a mother cell. The survivorship curve shows the percentage of the population of yeast cells that are viable plotted against replicative age. Average lifespan of the strains (expressed as mean number of buds removed ± SEM) was 11.1 ± 1.2 (n = 37) for *srs2*Δ-YEplac195 (*srs2*Δ-vector) and 13.3 ± 1.4 (n = 38) for *srs2*Δ-YEplac195-*SGS1* (*srs2*Δ-*SGS1*$^+$). Maximum lifespans recorded were 24 and 36, respectively.

DISCUSSION

Phenotypic overlap between *sgs1*Δ and *srs2*Δ mutants

Mutant *sgs1* and *srs2* strains have been shown to exhibit hyper-recombination, defective intra-S checkpoint responses and shortened replicative lifespans (13,18,27,37,38). The *sgs1*Δ mutant is also known to be sensitive to a variety of genotoxic agents (12,16,17,19,23). We report here that the *srs2*Δ mutant is similarly sensitive to MMS, hydroxyurea, camptothecin, mitoxantrone and 4-NQO, reinforcing the phenotypic overlap with the *sgs1*Δ mutant. In addition to its genotoxin sensitivity, the *sgs1*Δ mutant has a shortened replicative lifespan (22–24). We found that the *srs2*Δ mutant has a mean replicative lifespan ~45% of that of the wild-type strain. This is comparable with the average lifespan recorded for the isogenic *sgs1*Δ mutant (43%) (23), revealing a similar magnitude of shortened lifespan for the diploid *sgs1*Δ and *srs2*Δ mutants.

These findings are in agreement with a recent report documenting the phenotypes of the haploid *sgs1*Δ and *srs2*Δ mutants (38). In that work, it was revealed that the previous characterisation of premature ageing in the *sgs1* mutant as a phenocopy of normal ageing (24) was not entirely accurate. Instead, it was concluded that the short lifespan exhibited by both *sgs1*Δ and *srs2*Δ mutants was due to a combination of ageing-independent mitotic checkpoint arrest and accelerated normal ageing (38). To determine whether the increased G_2/M checkpoint arrest phenotype was also apparent in our diploid strains, we followed the approach of McVey *et al.* (38) and re-analysed our lifespan data for incidence of terminally budded phenotypes. This analysis was performed blind as our lifespan studies were completed before publication of their report. The morphology of *sgs1*Δ and *srs2*Δ terminal phenotypes revealed a higher percentage of budded mitotic intermediates than that observed for the isogenic wild-type strain (data not shown), thus confirming the findings of McVey *et al.* (38). As the severe phenotype of *sgs1srs2* double mutants can be suppressed by inactivation of Rad51, Rad52 or Rad57, it is likely that it results from unrestrained recombination (38), in keeping with the idea that both Sgs1 and Srs2 act to prevent such events during S phase.

Complementation of the *srs2*Δ mutant by *SGS1*

It has been suggested that the Sgs1 and Srs2 helicases may be functionally redundant, based on the fact that either single mutant is viable, but the double mutant has a severe growth defect (40). Although complete redundancy can be ruled out due to the abundance of phenotypes exhibited by each single mutant (see above), the similarities of these phenotypes are consistent with a degree of functional overlap. Our finding that *SGS1* can function as a multicopy suppressor of the MMS and hydroxyurea sensitivity of the *srs2*Δ mutant provides direct support for this notion. In contrast, high-copy *SRS2* was unable to complement genotoxin sensitivity of the *sgs1*Δ strain. One explanation of this finding is that Sgs1 performs some cellular function(s) that cannot be substituted by Srs2, despite the ability of Sgs1 to functionally replace Srs2. However, it is worth noting that, as reported previously (53), we found that higher levels of Srs2 caused detrimental effects in the wild-type strain.

Sgs1 is a large protein that, in addition to the RecQ helicase domain, has distinct domains required for interactions with Top2, Top3 and Rad51 (11,54–57). Furthermore, mutant alleles of *sgs1* have selective complementing ability in different phenotypic assays (17,23,50,51). Therefore, the domains of Sgs1 required for rescue of MMS sensitivity in the *sgs1*Δ background could potentially differ from those required for complementation of the *srs2*Δ mutant. To address this issue, we constructed libraries of *SGS1* mutant plasmids containing small in-frame insertions. Unsurprisingly, a large proportion of loss-of-function mutants for MMS resistance in the *sgs1*Δ background contained transposon insertions in or around the helicase domain of *SGS1*. This is consistent with reports that the helicase activity of Sgs1 is required for dealing with MMS-induced DNA damage (17,21,23,51,58). Some insertions were also found N-terminal to the helicase domain, mapping close to the interaction sites for Top2 and Top3. This supports the observation that the N-terminus of Sgs1 is essential for MMS resistance (17). Interestingly, no MMS loss-of-function alleles mapped to the C-terminus of Sgs1, where the Rad51 interaction occurs (57). Again, this is consistent with reports that much of the C-terminus of Sgs1 (the final 254 amino acids) is dispensable for MMS resistance (17,51). It was found that none of these *SGS1* loss-of-function plasmids tested could complement the *srs2*Δ mutant. Similarly, a point mutation causing inactivation of Sgs1 helicase activity (50) was unable to restore MMS resistance to the *srs2*Δ strain. This demonstrates that complementation of the *srs2*Δ mutant by high-copy *SGS1* requires domains of Sgs1 that are directly involved in processing MMS-induced DNA lesions.

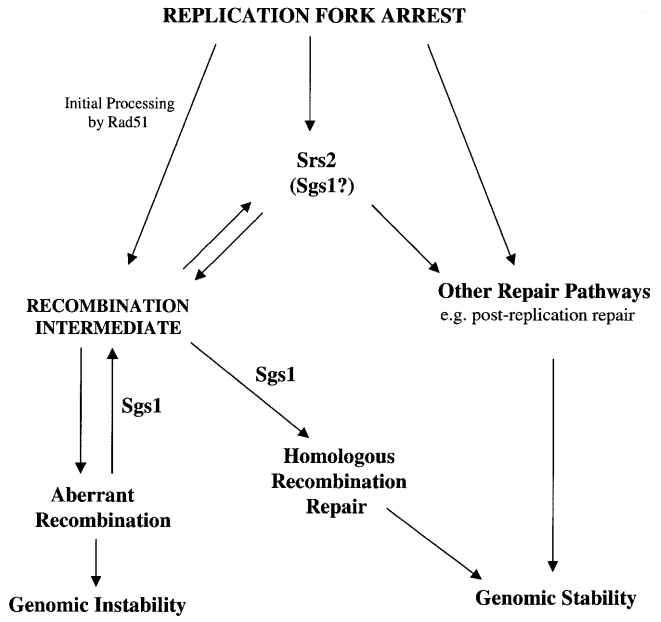

Figure 7. Model of Sgs1 and Srs2 function in maintaining genomic stability. A simplified diagram summarising the likely roles of Sgs1 and Srs2 is presented. It is probable that the short lifespan and genotoxin sensitivity in the *srs2*Δ mutant is due to unrestrained recombination. If levels of Sgs1 are limiting, the ability to antagonise aberrant recombination and promote conservative homologous recombination may thus be overwhelmed. Therefore, increased levels of Sgs1 may allow *srs2*Δ mutant cells to deal with this situation. Alternatively, it may be that higher levels of Sgs1 can directly replace Srs2 and process substrates that would normally be dealt with by Srs2.

When *SGS1* was over expressed in the *srs2*Δ mutant, no significant increase in the mean replicative lifespan was evident. This contrasts with the ability of *SGS1* overexpression to suppress MMS sensitivity of the *srs2*Δ mutant. One possible reason for this is that other important roles of Srs2 may still be lacking and contribute to the shortened mean replicative lifespan of the *srs2*Δ mutant. Alternatively, the beneficial effects of *SGS1* overexpression may be outweighed by detrimental effects associated with high levels of Sgs1. Several observations support the latter theory. First, *SGS1* overexpression can increase the maximum lifespan of the *srs2*Δ mutant by 50%. Secondly, *SGS1* overexpression has been reported to cause detrimental effects (18,24). Thirdly, *srs2*Δ cells over expressing *SGS1* actually show an increase in terminally budded morphologies as compared with control *srs2*Δ mutants containing empty vector (data not shown). It is remarkable that high-copy *SGS1* can extend maximum lifespan in the face of such deleterious effects.

Cellular functions of Sgs1 and Srs2

Proteins involved in recombinational repair (e.g. Rad52) and post-replication repair (e.g. Rad6 and Rad18) play important roles in processing DNA lesions induced by MMS, in an analogous manner to that proposed for the processing of UV-induced lesions (59). Srs2 is thought to channel lesions into post-replication repair pathways, whereas Sgs1 is thought to be more intimately involved in recombinational repair (2,57,60) (Fig. 7). The ability of Sgs1 overexpression to process MMS-induced DNA damage in the *srs2*Δ mutant implies that endogenous Sgs1 levels are normally limiting in the *srs2*Δ mutant. This effect of Sgs1 overexpression may be to increase the efficiency of Sgs1's normal physiological function(s), thereby reducing the requirement for Srs2-dependent repair pathways. Alternatively, it may be that Sgs1 is capable of directly replacing Srs2, by processing substrates that would normally be dealt with by Srs2.

A variety of data support the notion that the *sgs1*Δ and *srs2*Δ phenotypes are a consequence of unrestrained recombination (20). Consistent with this, mutations in the *RAD51* or *RAD52* genes can suppress the MMS sensitivity of the *srs2*Δ mutant (61), whereas overexpression of *RAD51* or *RAD52* enhances the MMS sensitivity of the *srs2*Δ mutant (62). Therefore, in the absence of Srs2, the Rad52-dependent recombination repair pathway is over-active (31), leading to aberrant processing of recombination intermediates and genomic instability (Fig. 7). However, it is worth noting that, in addition to its well-established anti-recombinase function, Srs2 has recently been claimed to promote recombination in some circumstances (63). The absence of Sgs1 would also be predicted to cause aberrant processing of recombination intermediates as not only are the later steps of the homologous recombination repair pathway impaired (57,64), but also aberrant recombination intermediates cannot be 'salvaged' by Sgs1 (14). This model is consistent with observations that the synthetic growth defect in *sgs1srs2* double mutants (40) can be rescued by mutation of genes involved in homologous recombination (20,38,39).

Although we favour the idea that Sgs1 overexpression rescues MMS sensitivity in the *srs2*Δ mutant by suppressing unrestrained recombination, it remains possible that the ability of Sgs1 overexpression to compensate for lack of Srs2 is unrelated to DNA recombination. This possibility is supported by the fact that inactivation of the homologous recombination pathway does not suppress the severe synthetic growth defects observed in the *S.pombe srs2*Δ*rqh1*Δ double mutant (29). Nevertheless, the fact that genetic interactions between *SGS1/rqh1*$^+$ and *SRS2* are evident in two such evolutionarily divergent species further supports the notion of functional overlap between these DNA helicases. It will be of interest to assess if overexpression of *rqh1*$^+$ can rescue any phenotypes of the *S.pombe srs2*Δ mutant.

Wider implications of functional overlap between DNA helicases

These findings may have potentially important ramifications for studies of the human RecQ helicases. First, the physiological effects of overexpression of Sgs1 may warrant further characterisation, as higher levels of both the BLM and WRN helicases have been reported to exist in transformed cells and tumour cells, relative to those found in normal cells (65,66). Furthermore, the finding that RecQ helicases can show partial functional redundancy with other DNA repair pathways may be significant for the understanding of the functions of RecQ family members in maintenance of genomic stability. In addition to the functional overlap between Sgs1 and Srs2 demonstrated here, an extra copy of *Ku70*, a DNA repair helicase, can partially rescue the phenotypes of *Drosophila* lacking the RecQ helicase *Dmblm* (47). This functional overlap of DNA repair pathways implies that defects in human RecQ helicases would have more severe effects under conditions where the redundant pathways are limiting. This may be important in

understanding the role of the human RecQ helicases in genomic stability, cancer and ageing.

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