Sgs1 binding to Rad51 stimulates homology-directed DNA repair in Saccharomyces cerevisiae

Lillian Campos-Doerfler *, Salahuddin Syed *† and Kristina H. Schmidt *‡

* Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, FL 33620, USA.
† Graduate Program in Cell and Molecular Biology, University of South Florida, Tampa, FL 33620, USA.
‡ Cancer Biology and Evolution Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA.
1 Present address: Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709, USA.
Running title: Sgs1-Rad51 stimulate recombination

Keywords: Sgs1, Rad51, homologous recombination, DNA damage, DNA repair

Corresponding author: Kristina Schmidt, Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, 4202 E. Fowler Avenue, ISA 2015, Tampa, FL 33620. Phone: (813) 974-1592. E-mail: kschmidt@usf.edu
ABSTRACT
Accurate repair of DNA breaks is essential for maintaining genome integrity and cellular fitness. Sgs1, the sole member of the RecQ family of DNA helicases in *Saccharomyces cerevisiae*, is important for both early and late stages of homology-dependent repair. Its large number of physical and genetic interactions with DNA recombination, repair and replication factors has established Sgs1 as a key player in the maintenance of genome integrity. To determine the significance of Sgs1 binding to the strand exchange factor Rad51 we have identified a single amino acid change C-terminal of the helicase core of Sgs1 that disrupts Rad51 binding. In contrast to an *SGS1* deletion or a helicase-defective *sgs1* allele, this new separation-of-function allele, *sgs1-FD*, does not cause DNA damage hypersensitivity or genome instability, but exhibits negative and positive genetic interactions with *sae2Δ, mre11Δ, exo1Δ, srs2Δ, rrm3Δ*, and *pol32Δ* that are distinct from those of known *sgs1* mutants. Our findings suggest that the Sgs1-Rad51 interaction stimulates homologous recombination (HR). Unlike *sgs1* mutations that impair the resection of DNA double-strand ends, however, negative genetic interactions of the *sgs1-FD* allele are not suppressed by *YKU70* deletion. We propose that the Sgs1-Rad51 interaction stimulates homologous recombination by facilitating the formation of the presynaptic Rad51 filament, possibly by Sgs1 competing with single-stranded DNA for replication protein A binding during resection.
INTRODUCTION

DNA double-strand breaks (DSBs) can be induced exogenously by DNA damaging agents or form endogenously if the replisome collapses at a nick in the template strand or encounters a physical barrier that blocks progression of the replisome, such as a bound protein, DNA adduct, interstrand crosslink, or unusual DNA structure. Cells can repair such DSBs by homologous recombination (HR) or non-homologous end-joining (NHEJ). In the event of a DSB, the NHEJ proteins Ku70/Ku80 (Ku) and HR proteins Mre11-Rad50-Xrs2 (MRX) initiate the repair process by binding to the DSB ends (MIMITOU and SYMINGTON 2010). NHEJ is preferred in G1 as there is no sister chromatid whereas HR is preferred during S phase and G2 (IRA et al. 2004; BARLOW et al. 2008; HUERTAS et al. 2008). In S phase, Ku and MRX bind to the DSB first and recruitment of Sae2 activates short range resection, removing Ku and MRX and leaving a small 3’ single-stranded DNA (ssDNA) overhang (TRUJILLO et al. 2003; MIMITOU and SYMINGTON 2008; MIMITOU and SYMINGTON 2010; NICOLETTE et al. 2010; NIU et al. 2010; CANNAVO and CEJKA 2014). These trimmed DNA ends are then more extensively resected by the 5’- 3’ exonuclease Exo1 or by Dna2 nuclease (MIMITOU and SYMINGTON 2008; ZHU et al. 2008; LEVIKOVA et al. 2017). Exo1 and Dna2 differ in that Exo1 can degrade the 5’ strand in double-strand DNA (dsDNA) whereas Dna2 requires the Sgs1 helicase to unwind the dsDNA to provide ssDNA on which Dna2 can act (CANNAVO et al. 2013; LEVIKOVA et al. 2017). Long-range resection by Sgs1/Dna2 and Exo1 is redundant, with loss of both activities resulting in a severe resection defect and mutagenic repair (MIMITOU and SYMINGTON 2008; ZHU et al. 2008; DOERFLER et al. 2011). Sgs1 also
interacts with Mre11 and this may help to recruit Sgs1/Dna2 to the DSB after initial resection (LISBY et al. 2004; CHIOLO et al. 2005).

Rad52, an essential HR factor in budding yeast, then allows a recombinogenic Rad51 filament to assemble on the replication protein A (RPA) coated 3’-overhang (NEW et al. 1998; SONG and SUNG 2000). Regulation of HR at this stage relies on the antirecombinase Srs2, which can disassemble Rad51 filaments (KREJČI et al. 2003; VEAUTE et al. 2003; LIU et al. 2011). If Rad51-mediated homology search is successful the 3’ end of the invading strand is extended by DNA synthesis. In classic DSB repair (DSBR) the second end of the DNA break is also captured to form a double-Holliday Junction (dHJ), which can either be resolved by endonucleases Mus81/Mms4 or Yen1 to produce both crossovers and noncrossovers, or the HJs are converged and decatenated by the Sgs1/Top3/Rmi1 complex, resulting in only noncrossovers (WU and HICKSON 2003; CEJKA et al. 2010b; CEJKA et al. 2012).

Sgs1 is a member of the highly conserved family of RecQ-like DNA helicases, which interact with a large number of proteins with functions in genome maintenance. Sgs1 not only interacts with Dna2, Mre11, Top3/Rmi1, but also contains acidic regions in its long unstructured N-terminal tail that are required for binding the ssDNA binding protein RPA (GANGLOFF et al. 1994; MULLEN et al. 2005; UI et al. 2005; HEGINAUER et al. 2012; KENNEDY et al. 2013). Rad53 kinase, Top2 topoisomerase and the nucleotide excision repair factor Rad16 have also been shown to physically interact with the N-terminal tail of Sgs1, whereas Rad51 and Mlh1 binding has been narrowed down to the region C-terminal of the helicase core (WATT et al. 1995; FRICKE et al. 2001; SAVIT et al. 2001; WU et al. 2001; DHERIN et al. 2009; HEGINAUER et al. 2012).
Lack of Sgs1 results in increased sensitivity to DNA damaging agents, shortened life span, missegregated chromosomes, and moderate accumulation of gross-chromosomal rearrangements (GCRs), including characteristic recurrent translocations between short homologous, but non-allelic, sequences (Sinclair et al. 1997; Mullen et al. 2000; Myung et al. 2001; Fricke and Brill 2003; Schmidt et al. 2010a). Cells lacking Sgs1 exhibit growth defects or die in the absence of structure-specific endonucleases Mus81/Mms4 and Slx1/4 that resolve recombination intermediates and stalled replication forks, the HR factors MRX or Sae2, the antirecombinase Srs2, or the Rrm3 helicase, which regulates replisome progression (Lee et al. 1999; Mullen et al. 2001; Fricke and Brill 2003; Schmidt and Kołodner 2004; Torres et al. 2004; Pan et al. 2006; Syed et al. 2016).

This multitude of physical and genetic interactions has established Sgs1 as a key player in the maintenance of genome integrity. The molecular basis and functional significance of some of the physical interactions for HR are increasingly well understood, especially the interaction of Sgs1 with Top3/Rmi1 in dHJ dissolution, with Dna2 in DSB resection, and with mismatch repair factors in the suppression of mitotic and meiotic homeologous recombination (Mullen et al. 2000; Wang and Kung 2002; Spell and Jinks-Robertson 2004; Amin et al. 2010; Kennedy et al. 2013; Levikova et al. 2017).

Here, to understand the role of the physical interaction of Sgs1 with Rad51 in homology-dependent DNA repair, we set out to identify a separation-of-function allele of SGS1 that disrupts Sgs1-Rad51 binding and to characterize the genetic interactions of this sgs1 allele in cells with replication-dependent DNA lesions.
MATERIALS AND METHODS

**Yeast Strains and Media:** Yeast strains were derived from S288C strain KHSY802 (MATa, ura3-52, trp1Δ63, his3Δ200, leu2Δ1, lys2Bgl, hom3-10, ade2Δ1, ade8, hxt13::URA3). SGS1 mutant alleles for amino acid changes K706A (sgs1-HD, pKHS787) and F1192D (sgs1-FD, pKHS786) were generated by site-directed mutagenesis (QuikChange, Stratagene) of wildtype SGS1 in pKHS360 (pRS405-SGS1.TRP1) and integrated in the chromosome under control of the endogenous SGS1 promoter by LiAc-mediated transformation as described (GIETZ and WOODS 2006). Haploid strains with multiple mutant alleles were obtained by sporulating diploids heterozygous for the desired mutations and genotyping spores on selective media or PCR. All yeast strains used in this study are listed in Supplementary Table S1. Yeast were grown at 30° in yeast extract, peptone, and dextrose (YPD) or synthetic complete (SC) media as previously described (MIRZAEI et al. 2011). Solid media was supplemented with 20 g/l agar (US Biological).

**DNA-damage sensitivity assays:** Sensitivity of yeast cells in exponential growth phase to HU and MMS was tested by spot assays as previously described (MIRZAEI and SCHMIDT 2012). Briefly, cell cultures were grown in liquid YPD medium OD_{600}=0.5 and ten-fold serial dilutions were spotted on YPD containing the indicated concentration of HU (US Biological) or MMS (Sigma-Aldrich). Images of colony growth were acquired every 24 hours for five days of incubation at 30° with a GelCam 315 CCD camera mounted on a Gel-Doc IT Imaging system (UVP).
**Gross-Chromosomal Rearrangements (GCR) Assay:** Cells with GCRs were identified by simultaneous inactivation of *CAN1* and *URA3* on chromosome V indicated by resistance to canavanine and 5-fluoro-orotic acid (Can<sup>+</sup> 5-FOA<sup>+</sup>). Cultures were grown for two days in at least 10 ml of YPD media. Viable cell counts were determined by plating dilutions on YPD agar plates, and cells with GCRs were identified by plating 0.25 ml-15 ml on synthetic media lacking arginine and uracil and supplemented with 60 mg/l canavanine (Sigma) and 1 g/l 5-fluoro-orotic acid (US Biological). Rate of accumulating GCRs were calculated as previously described (SCHMIDT et al. 2010b).

**Mutator Assays and Mutation Spectrum Analysis:** Rates of accumulating mutations at the *CAN1* locus or reversion mutations in the *hom3-10* or *lys2-Bgl* alleles were determined by fluctuation analysis by the method of the median (LEA and COULSON 1949) in at least fourteen cultures from at least two different isolates as previously described (REENAN and KOLODNER 1992). Cultures were grown overnight in 3 - 6 ml of YPD media. Viable cell counts were determined by plating dilutions on YPD agar plates and 250 µl - 6 ml were plated on synthetic media lacking arginine and supplemented with 60 µg/ml canavanine for selection of inactivation of *CAN1*, or on SC media lacking threonine or lysine to select for threonine (*hom3-10*) or lysine (*lys2-Bgl*) revertants, respectively. Median rates are reported with 95% confidence intervals (NAIR 1940). The spectrum of inactivating mutation at the *CAN1* locus was determined by sequence analysis of *CAN1* from canavanine-resistant colonies using primer pairs that anneal 50 bp upstream and 43 bp downstream of the *CAN1* ORF.
**Gap repair assay:** Crossover and noncrossover outcomes in a gap repair assay were determined as previously described (Mitchel *et al.* 2010). The can1::his3Δ3' allele from plasmid pSR800 was inserted at the chromosomal CAN1 locus of wildtype, sgs1Δ and sgs1-FD cells. Cells were then transformed with linearized pSR987, which contains the his3 template for gap repair and a counter-selectable URA3 marker. Plasmids pSR800 and pSR987 were kindly provided by Sue Jinks-Robertson (Duke University). His+ colonies were selected on synthetic complete media lacking histidine (SC-His). Whether His+ colonies had formed by crossover or noncrossover events was determined by their ability to grow on agar plates containing 5-fluoro-orotic acid, indicating loss of URA3. Briefly, colonies were first grown in the absence of histidine and then in nonselective media (either liquid YPD or as patches on YPD agar) before being spotted or replica- plated on agar plates containing 1 g/l 5-fluoro-orotic acid. Fully grown spots/patches were scored as noncrossovers and those with few or no colonies were scored as crossovers. Over 140 His+ colonies for each strain from two independent can1::his3-0,Δ3' isolates of the wildtype strain, and sgs1Δ and sgs1-FD mutants were analyzed.

**Tetrad analysis:** Diploid strains for tetrad dissection were derived from S288C strains provided by Richard Kolodner (UC San Diego) and are listed in Supplementary Table S1. To generate the diploid KHSY4810, heterozygous for sgs1Δ, rad52Δ, and exo1Δ mutations, RDKY5290 was crossed to KHSY4805 (an exo1Δ rad52Δ spore obtained from a cross between RDKY2614 and RDKY2710). RAD59 and RAD51 deletions were obtained by HR-mediated integration of a selectable marker at these loci in RDKY2666 using the LiAc method (Gietz and Woods 2006) and diploids heterozygous for sgs1Δ,
exo1Δ, and rad51Δ or rad59Δ were obtained by crossing as described above. For tetrad
dissections, diploids were grown overnight in YPD at 30°C and starved of nitrogen in
0.1% potassium acetate. Asci were briefly incubated with zymolase and dissected on
YPD agar plates using a micromanipulator mounted on an Axioscop 40 (Zeiss). YPD
plates were incubated for 4 days at 30°C and spore germination and colony growth were
documented at 24 hour intervals with a CCD camera mounted on a GelDoc-IT Imager
(UVP).

**Pull-Down Assay and Western Blotting:** Plasmid pKHS657, expressing GST-Sgs1<sup>647-1447</sup>, was created by inserting the last 2400 bp of SGS1 into pGEX-6p-2 (GE Healthcare)
using BamHI and XhoI restriction sites. Stop codons and F1192A and F1192D mutations
were introduced at the indicated positions by site-directed mutagenesis (Quikchange,
Agilent Genomics). Sgs1 fragments were expressed in *E. coli* BL21 (DE) in LB media
(10 g/l tryptone, 5 g/l NaCl, 5 g/l Yeast extract) supplemented with 1.5 µg/ml ampicillin
for 3 hours in the presence of 1 mM IPTG. Cells were resuspended in 100 µl GST buffer
(125mM Tris, 150mM NaCl, pH 8.0) plus HALT protease inhibitor cocktail (Pierce),
lysed using glass beads with a BeadBeater (Biospec Products, Inc.) at 4°C and lysate
cleared by centrifugation at 14000 rpm for 10 min at 4°C. Lysate was treated with
benzonase (Sigma) and 1mg of lysate was added to glutathione magnetic beads (Pierce)
and incubated for 1h at 4°C before beads were washed three times with GST buffer.
Similarly, yeast cells expressing endogenous levels of VSV-tagged Rad51 (Open
Biosystems) were resuspended in Rad51 buffer (50 mM Tris, pH 7.5, 0.01% NP-40, 5
mM β-glycerol phosphate, 2 mM magnesium acetate, 120 mM NaCl) with HALT
protease inhibitor cocktail (Pierce), lysed with glass beads in a BeadBeater and cleared by
centrifugation at 14000 rpm for 20 min at 4°C. Lysate was treated with benzonase (Sigma) and 10 mg of lysate were incubated with Sgs1-bound magnetic beads for 120 min at room temperature while rotating. Beads were washed five times with Rad51 buffer plus HALT protease inhibitor cocktail (Pierce) and then boiled in Laemmle buffer (Bio-Rad) for 10 min. The eluate was separated by 10% SDS-polyacrylamide gel electrophoresis. Sgs1 fragments and Rad51 were detected by Western blotting using monoclonal antibodies against GST (Covance) and VSV (Sigma) epitopes.

Data and reagent availability: Yeast strains are available upon request. Table S1 contains a list of yeast strains used in this study and detailed genotype descriptions.

RESULTS

Rad51 binds to the loop that connects the helicase core of Sgs1 to the HRDC domain: SGS1 and RAD51 are epistatic and the gene products interact physically (Wu et al. 2001; Torres et al. 2004). Using a yeast-two-hybrid assay, the physical interaction with Rad51 was previously mapped to the last 469-residues of Sgs1 (residues 978-1447). This region flanks the ATPase domain and contains the conserved RQC domain, which is essential for the helicase activity of Sgs1, as well as other conserved sites, including the HRDC domain and an Mlh1 binding site (Pedrazzi et al. 2001; Wu et al. 2001). Thus, disrupting Rad51 binding by deleting this 469-residue region disrupts multiple other Sgs1 functions. Therefore, to enable elucidation of the biological importance of the interaction between Sgs1 and Rad51 for HR, we sought to identify a separation-of-function mutation
in Sgs1 that specifically disrupts Rad51 interaction, but leaves other functional sites intact.

To narrow down the Rad51 binding region we purified fragments of Sgs1 as GST-fusions from *E. coli* and tested their ability to pull-down endogenous VSV-epitope tagged Rad51 from yeast whole cell extract. We determined that deleting up to 240 C-terminal residues of Sgs1 did not impair its ability to interact with Rad51, whereas a deletion of 260 residues abolished it (Figure 1A and 1B). This critical 20-residue region maps to residues 1187 to 1207 immediately C-terminal of the WH-domain and contains a phenylalanine at position 1192. Mutating this hydrophobic residue to aspartic acid (F1192D) disrupted Rad51 binding, whereas mutating it to alanine did not have an effect (Figure 1B).

Unlike loss of Sgs1 helicase activity, loss of Sgs1-Rad51 binding does not cause DNA damage sensitivity and genome instability in haploid cells: To determine how the loss of Rad51 binding affects Sgs1 function *in vivo* we integrated the *sgs1-F1192D* allele (hereafter *sgs1-FD*) at the chromosomal locus under control of the *SGS1* promoter. Expression levels of *sgs1-FD* were similar to those of wildtype Sgs1 (Figure 1C). Unlike an *SGS1* deletion, the *sgs1-FD* mutation did not increase genome instability (Table 1) or sensitivity to hydroxyurea (HU) (Figure 1D). Since the *sgs1-FD* mutation is disrupting a link between Sgs1 and HR, we also tested its effect on DNA-damage sensitivity in diploids, which depend more strongly on HR for repair of DNA breaks than haploids (Frank-Vaillant and Marcand 2001; Li and Tye 2011). Diploids were indeed more
Sensitive to MMS if they were homozygous for the *sgs1-FD* mutation (Figure 1D), indicating a mild DNA repair defect in the *sgs1-FD* mutant.

**Rad51 binding to Sgs1 is required for normal growth, DNA damage tolerance, and genome stability in the absence of Sae2, but not Mre11:** To identify Sgs1 functions that are impacted by its binding to Rad51, we first investigated genetic interactions between *sgs1-FD* and HR genes. In HR, Sgs1 acts in addition to Exo1 in the resection of DSBs after their initial nucleolytic processing by Sae2/MRX (Mimitou and Symington 2008). In cells lacking *SGS1*, deletion of *EXO1* causes a fitness defect and one of the largest known synergistic increases in genomic instability (>500-fold) (Gravel et al. 2008; Doerfler et al. 2011; Doerfler and Schmidt 2014) whereas a deletion of *SAE2* or *MRE11* is synthetically lethal with *sgs1A* (Shor et al. 2002; Pan et al. 2006). These reported phenotypic similarities between Sae2 and Mre11 deficiency, however, did not apply to the *sgs1-FD* mutant. The *sgs1-FD* mutation caused a significant fitness defect in the *sae2A* mutant, but had no detrimental effect on the growth of the *mre11A* mutant (Figure 2A). The *sgs1-FD* allele also increased hypersensitivity of the *sae2A* mutant to HU and MMS, but exhibited a wildtype phenotype in the *mre11A* mutant (Figure 2B and 2C). Moreover, the *sgs1-FD* allele led to a synergistic (25-fold) increase in the GCR rate in the *sae2A* mutant, but had no effect on the accumulation of genome rearrangements in the *mre11A* mutant (Figure 2D).

In the current model of DSB end processing, MRX and Sae2 bind to the unprocessed ends, trimming off a few nucleotides and causing their own release from the
DNA (MIMITOU and SYMINGTON 2008; ZHU et al. 2008). These trimmed ends are poor substrates for Ku binding, but good substrates for extensive nucleolytic processing by Exo1 and Sgs1/Dna2 to produce the long 3’ terminated overhangs for Rad51-mediated homology search (MIMITOU and SYMINGTON 2008; ZHU et al. 2008). When initial trimming and long-range resection are impaired due to absence of Sae2 and Sgs1 cells die (TONG et al. 2001; OOI et al. 2003). However, these cells are rescued by deleting YKU70, suggesting that preventing Ku from binding to the DSB ends makes them accessible to the alternative, Exo1-mediated pathway for long-range resection, thus bypassing the requirement of Sae2 for removal of Ku and that of Sgs1 for long-range resection (MIMITOU and SYMINGTON 2010). Based on this finding, any sgs1 mutation that causes a resection defect and a synthetic growth defect with sae2Δ should be suppressed by deletion of YKU70. Indeed, the defects of the sgs1-D664Δ mutant, which include a severe fitness defect with sae2Δ and a resection defect, are bypassed in cells lacking Ku (BERNSTEIN et al. 2009; BERNSTEIN et al. 2013). However, we observed that neither the severe fitness defect of the sgs1-FD sae2Δ mutant nor its DNA damage sensitivity were suppressed by deleting YKU70 (Figure 2C), suggesting that the sgs1-FD mutation does not cause a resection defect.

To test the possibility that the requirement of Sae2 in the sgs1-FD mutant was related to an MRX-related function of Sae2, we next deleted MRE11 in the sae2Δ sgs1-FD mutant. We observed that the mre11Δ mutation suppressed the growth defect and the associated HU/MMS sensitivity of the sgs1-FD sae2Δ mutant to levels observed in the mre11Δ sae2Δ mutant (Figure 2A and 2C), indicating that unlike in the sgs1Δ mutant
MRX is not required in the \textit{sgs1-FD} mutant and that, in fact, the inability to remove MRX from the DSB ends is toxic in the \textit{sgs1-FD} mutant.

\textit{sgs1-FD} increases genome instability and DNA damage hypersensitivity in cells lacking Exo1: The need in the \textit{sgs1-FD} mutant for SAE2 prompted us to further investigate the requirement of \textit{EXO1}, which cooperates with Sgs1/Dna2 during more extensive resection of DSB after Sae2. The \textit{exo1Δ} mutant is mildly sensitive to high concentrations of MMS, but not HU (Doerfler and Schmidt 2014). The \textit{sgs1-FD} allele increased the sensitivity of the \textit{exo1Δ} mutant to MMS and caused sensitivity to 200 mM HU, but remained far below the effect of an \textit{SGS1} deletion (Figure 3A). The \textit{sgs1-FD} allele also caused a significant (6-fold) increase in the accumulation of genome rearrangements in the \textit{exo1Δ} mutant (Figure 2D) albeit this, too, was much milder than the 500-fold increase in GCR accumulation previously reported for the \textit{SGS1} deletion (Gravel et al. 2008; Doerfler et al. 2011).

Suppression of the severe growth defect of the \textit{top3Δ} mutant by the \textit{sgs1-FD} mutation: In addition to interacting with Dna2 and RPA during DSB resection, Sgs1 forms a complex with Top3/Rmi1 to dissolve dHJs (Gangloff et al. 1994; Chang et al. 2005; Mullen et al. 2005). In vitro, Top3 also stimulates Sgs1 activity in DSB resection and resolves protein-bound D-loops (Cejka et al. 2010a; Fasching et al. 2015). Both, deletion of \textit{SGS1} or loss of Sgs1 helicase activity, suppress the severe growth defect of the \textit{top3Δ} mutant, which has been interpreted to mean that Sgs1 produces HR.
intermediates that then require Top3 for dissolution (Gangloff et al. 1994). We found that the sgs1-FD allele, too, suppressed the severe slow growth phenotype of the top3Δ mutant to a similar extent as the sgs1Δ or sgs1-HD alleles and improved growth during exposure to HU or MMS (Figures 3A and 3B). This suggests that the interaction between Sgs1 and Rad51 drives the formation of recombination intermediates and thereby significantly contributes to the severe growth defect of the top3Δ mutant.

Since Sgs1 and its interaction with Top3/Rmi1 are important for the dissolution of recombination intermediates, we also tested the effect of the sgs1-FD mutation on crossover and noncrossover formation in a gap repair assay (Welz-Voegele and Jinks-Robertson 2008). Deletion of SGS1 led to an increase in the fraction of crossovers, which is consistent with previous findings (Welz-Voegele and Jinks-Robertson 2008), whereas the sgs1-FD mutant exhibited a ratio of crossovers to noncrossovers similar to wildtype cells (Figure 4C). This suggests that the ability of Sgs1/Top3/Rmi1 to dissolve HJs is largely unaffected by the FD mutation in Sgs1.

Opposite effects of the sgs1-FD mutation on the DNA-damage sensitivity of srs2Δ and rrm3Δ mutants: Srs2 acts as an inhibitor of HR through its ability to disrupt the Rad51 presynaptic filament (Krejci et al. 2003; Veaute et al. 2003). In the absence of Srs2, cells become hyper-recombinogenic, hypersensitive to exogenous DNA damage and replication stress, as well as dependent on Sgs1 for viability (Lee et al. 1999; Krejci et al. 2003; Veaute et al. 2003). The negative genetic interactions of the sgs1-FD allele with sae2Δ and exo1Δ mutations, and the positive interaction with top3Δ suggest that
*sgs1-FD* is a hypo-recombination allele of *SGS1*. To further explore this possibility, we introduced *sgs1-FD* into the *srs2Δ* mutant, which we expected to benefit from a reduction in HR. Indeed, in stark contrast to a deletion of *SGS1* or loss of Sgs1 helicase activity, which are both lethal to *srs2Δ* cells, the *sgs1-FD* allele had no detrimental effect on the growth of *srs2Δ* cells and in fact suppressed the hypersensitivity of *srs2Δ* cells to HU and MMS by more than 10-fold (Figure 3B), consistent with *sgs1-FD* being a hypo-recombination allele.

We also investigated the importance of the Sgs1-Rad51 interaction in the *rrm3Δ* mutant. Replisomes pause frequently at many sites throughout the genome when the Rrm3 helicase is absent, generating DNA lesions that are substrates for Sgs1 and Rad51-dependent repair (*IVESSA et al. 2002; SCHMIDT and KOLODNER 2004; TORRES et al. 2004*). Like a deletion of *SRS2*, deletion of *RRM3* causes a severe growth defect in *sgs1Δ* cells that can be suppressed by deleting *RAD51* (*SCHMIDT and KOLODNER 2004; TORRES et al. 2004*). The *sgs1-FD* mutation did not cause a growth defect in *rrm3Δ* cells; however, cells became highly sensitive to both HU and MMS (Figure 3B). Despite the increased DNA damage sensitivity, the *rrm3Δ sgs1-FD* mutant did not accumulate genome rearrangements (Figure 3C, Table 1) in contrast to the *rrm3Δ sgs1Δ* mutant (*SCHMIDT et al. 2006*). This genetic interaction between *rrm3Δ* and a hypomorphic allele of *SGS1* further underscores the strong dependence of the repair of the replication-associated DNA lesions in *rrm3Δ* cells on homology-directed replication fork restart and rescue (*SYED et al. 2016*).
Sgs1-Rad51 interaction promotes large deletions and contributes to DNA damage hypersensitivity of cells lacking Pol32: In the absence of POL32, which connects polymerase δ to the processivity factor PCNA, DNA replication is inefficient and prone to pausing and mutations (Burgers and Gerik 1998; Huang et al. 2002; Johansson et al. 2004). Since an SGS1 deletion causes a fitness defect and increased HU and MMS sensitivity in the pol32Δ mutant, we decided to assess the effect of the sgs1-FD allele in this mutant. Surprisingly, we found that the sgs1-FD mutation had the opposite effect of the SGS1 deletion and the helicase-defective sgs1-HD allele, suppressing the HU hypersensitivity of the pol32Δ mutant (Figure 5A). Since the HU hypersensitivity of the pol32Δ mutant is also suppressed by deletion of EXO1 (Doerfler and Schmidt 2014), we next tested the combined effect of exo1Δ and sgs1-FD mutations on HU sensitivity of the pol32Δ mutant (Figure 5B). Instead of suppression, however, sensitivity to HU and MMS increased even at low drug concentrations, suggesting that Exo1 and the Sgs1-Rad51 interaction cooperate in a pathway that is required in the absence of Pol32. Since both Sgs1 and Exo1 act in DSB end processing to initiate HR, we investigated the effect of a RAD51 deletion on the HU/MMS sensitivity of the pol32Δ mutant and observed a strong increase in sensitivity (Figure 5B). This suggests that pol32Δ cells depend on a Rad51-dependent HR pathway for the survival of replication stress and that Exo1 and the Sgs1-Rad51 interaction independently promote this pathway.

The accumulation of large deletions between short repeats in CAN1 or other genomic loci is characteristic of pol32Δ cells and has been explained by an increased propensity of the nascent strands to dissociate from their templates as a result of frequent
pausing, followed by error-prone reannealing (HUANG et al. 2002). When we combined the pol32Δ mutation with the sgs1-FD mutation there was no significant change in the mutation rate at CAN1 (Supplemental Table S2), but the rate of large deletions was reduced 4-fold. This reduction is similar to the 6-fold reduction when SGS1 is deleted, suggesting that the interaction of Sgs1 with Rad51 contributes to the formation of large deletions in the absence of Pol32 (Figure 5C). Deletion of EXO1 in the pol32Δ mutant had the opposite effect, increasing the rate of large deletions 4-fold (Figure 5C). These observations indicate that, in contrast to their cooperative roles in DSB end resection, Exo1 and Sgs1 have opposite effects at impaired replication forks. For example, Exo1 might prevent deletions by degrading the nascent DNA strands that are prone to DNA slippage, whereas Sgs1 and the Sgs1-Rad51 interaction might help generate deletions by facilitating slipped strand mispairing at downstream repeats.

**Rad52/Rad59-mediated DNA repair, but not Rad51, is essential for cells with compromised DNA resection due to lack of Sgs1 and Exo1:** Cells lacking Sgs1 and Exo1 show minimal resection of DSBs and accumulate GCRs at an extreme level (GRAVEL et al. 2008; MIMITOU and SYMINGTON 2008; DOERFLER et al. 2011). Because of the failure to sufficiently resect DSB ends we expected that DSBs would not be suitable for repair by HR. Surprisingly, however, we found that deleting RAD52 was lethal in the sgs1Δ exo1Δ mutant, and deletion of RAD59 caused an extreme growth defect (Figure 6A). In contrast, deleting RAD51 caused only a mild fitness defect (Figure 6A), consistent with the ability of other groups to readily obtain and characterize the
When we analyzed the effect of HR mutations on chromosome rearrangements in the sgs1Δ exo1Δ mutant, we found that deleting RAD51 significantly suppressed (3.1-fold) their accumulation (Figure 6C, Table 2). We also analyzed GCR formation in the sgs1Δ exo1Δ rad59Δ mutant. Because of the severe growth defect of this mutant and the associated risk of obtaining suppressors during prolonged propagation, we set up all cell cultures from colonies immediately after they formed from meiotic products of the heterozygous diploid. In contrast to the decrease upon RAD51 deletion, RAD59 deletion doubled (2.4-fold) the GCR rate of sgs1Δ exo1Δ cells (Figure 6C, Table 2).

The dramatic decrease in viability of the sgs1Δ exo1Δ mutant upon RAD59 and RAD52 deletion suggests that a Rad59-dependent HR pathway repairs DNA lesions in this mutant. The decrease in GCR formation upon RAD51 deletion and the opposite effect of a RAD59 deletion further suggest that Rad51 and Rad59 compete for repair of these incompletely processed DNA lesions in the sgs1Δ exo1Δ mutant, and that repair by Rad51, but not Rad59, is mutagenic.

Interestingly, we observed that the effect of RAD51 and RAD59 deletions on the accumulation of GCRs is the same in sgs1Δ cells with EXO1 intact as in cells with EXO1 deleted; that is, a RAD51 deletion led to a significant (5.1-fold) decrease in the GCR rate of sgs1Δ cells (versus 3.1-fold decrease in sgs1Δ exo1Δ) and RAD59 deletion to a significant (1.8-fold) increase (versus a 2.3-fold increase in sgs1Δ exo1Δ) (Figure 6B and 6C, Table 2). Essentially, deleting EXO1 increased genome instability ~ 700-fold, but had
no effect on the genetic interactions between sgs1Δ, rad51Δ and rad59Δ (compare the last three columns in Figures 6B with 6C).

**DISCUSSION**

We have identified a novel separation-of-function mutant of Sgs1 (sgs1-FD) that fails to interact with Rad51, but does not cause the severe sensitivity to DNA-damaging agents seen in cells lacking Sgs1 or expressing helicase-defective Sgs1. Novel positive and negative genetic interactions between this sgs1-FD allele and mutations in genes with roles in HR (mre11Δ, sae2Δ, srs2, exo1Δ, top3Δ) or replisome progression (pol32Δ, rrm3Δ) suggest that the physical interaction between Sgs1 and Rad51 stimulates homology-dependent DNA repair.

We observed the strongest genetic interaction of the sgs1-FD allele with a SAЕ2 deletion (Figure 2). Sae2 removes MRX from DSB ends and prevents Ku binding, making the DSB accessible to extensive resection by Sgs1/Dna2 and Exo1 (Mimitou and Symington 2010). YKU70 deletion suppresses resection defects in cells that lack Sae2 and Sgs1 activities by allowing the alternative Exo1 pathway access to the DSB ends for resection (Bernstein et al. 2009; Mimitou and Symington 2010; Shim et al. 2010; Bernstein et al. 2013). In addition to suppressing the DNA-damage sensitivity and fitness defect of the sgs1Δ sae2Δ mutant, the resection-defective sgs1-D664Δ mutant was found to benefit from deleting YKU70 (Bernstein et al. 2009; Bernstein et al. 2013). In
contrast, deleting YKU70 had no effect on the sgs1-FD sae2Δ mutant identified here, indicating that the sgs1-FD mutant does not benefit from increased Exo1 activity at DSBs and, thus, that sgs1-FD is proficient for resection.

Surprisingly, even though Sae2 acts with MRX in the initial processing step, and sgs1Δ is synthetically lethal with both sae2Δ and mre11Δ, disruption of the Sgs1-Rad51 interaction was not detrimental to mre11Δ cells. In fact, the MRE11 deletion suppressed the detrimental effects of the sgs1-FD mutation in sae2Δ cells, suggesting that the Sae2 function that is critical in sgs1-FD cells is the removal of MRX from DSB ends. If MRX stays bound, the DNA-damage checkpoint is activated and oligomers of the Rad9 checkpoint adaptor accumulate nearby (USUI et al. 2001; CLERICI et al. 2006; CHEN et al. 2015; PUDDU et al. 2015). Disrupting the DNA damage checkpoint alleviates the requirement for Sae2 at DSBs (FERRARI et al. 2015). Although this suggests that Sgs1 can compensate for the lack of initial resection by Sae2, more extensive resection and Rad51 filament formation are still impaired by MRX stuck on the DSB ends (FERRARI et al. 2015; GOBBINI et al. 2015). Recent findings have indicated that Sgs1 can eventually remove MRX and analysis of the sgs1-D664Δ mutant linked this ability to long-range resection by Sgs1 (BERNSTEIN et al. 2013; FERRARI et al. 2015). Because of the resection defect of the sgs1-D664Δ mutant, defects of the sgs1-D664Δ sae2Δ mutant could be suppressed by deleting YKU70. That the YKU70 deletion had no effect on the sgs1-FD sae2Δ mutant indicates that the suppression by MRE11 deletion is not related to a resection defect in the sgs1-FD mutant. We propose, therefore, that the disruption of Sgs1-Rad51 interaction by the sgs1-FD mutation reduces the efficiency of Rad51
filament formation, and thus repair by HR. Removing \textit{MRE11} from the DSB ends and, consequently, preventing Rad9 accumulation around the DSB ends could compensate for this deficiency in the \textit{sae2Δ sgs1-FD} mutant by increasing the efficiency of long-range resection due to increased access of sgs1-FD/Dna2 to the DSB ends. Thus, when DNA end processing is impaired because of the lack of Sae2, and persistent MRX binding and resulting checkpoint activation inhibit Sgs1/Dna2 function in resection, HR increasingly depends on the stimulation of Rad51 filament formation by Sgs1.

The requirement of Sae2 in the \textit{sgs1-FD} mutant could also point to some overlap between functions of Sgs1 and Sae2 during early steps of HR, such as initial resection of DSB ends.

All other genetic interactions of the \textit{sgs1-FD} allele investigated here are also in agreement with a role of the Sgs1-Rad51 interaction in stimulating HR, such as the positive interactions of the \textit{sgs1-FD} mutation with \textit{top3Δ, pol32Δ and srs2Δ} and the negative interaction with \textit{rrm3Δ}. Especially the suppression of the DNA damage sensitivity of the \textit{srs2Δ} mutant strengthens our hypothesis that the Sgs1-Rad51 interaction stimulates Rad51 filament formation. Based on the ability of Srs2 to disassemble Rad51 filaments (\textit{KREJC1 et al. 2003}), suppression of \textit{srs2Δ sgs1Δ} synthetic lethality by \textit{RAD51} deletion (\textit{GANGLOFF et al. 2000}) could be interpreted in two ways: either Sgs1 acts like Srs2 by disassembling presynaptic Rad51 filaments, or Sgs1 in complex with Top3/Rmi1 is needed to dissolve the accumulating recombination intermediates that overwhelm the cell because Rad51 filaments are no longer disrupted by Srs2. Our findings suggest the second explanation to be true; if the Sgs1-Rad51
interaction indeed promoted the disassembly of presynaptic Rad51 filaments, then the disruption of the Sgs1-Rad51 interaction by the \textit{sgs1-FD} mutation would not have suppressed the DNA-damage sensitivity of the \textit{srs2Δ} mutant (Figure 3B).

Thus, taken together, the genetic interactions of the \textit{sgs1-FD} allele are distinct from those of the \textit{sgs1Δ} and helicase-defective \textit{sgs1-hd} alleles (Figure 7B) and consistent with a model (Figure 7A) whereby Sgs1 is not only responsible for the resection of DSB ends and the formation of ssDNA overhangs but, through interaction with Rad51, promotes HR by stimulating formation of the Rad51 presynaptic filament. As DNA ends are resected, RPA binding to the newly formed ssDNA overhangs limits the initiation of the Rad51 filament. Rad52 is essential to overcome this limitation and form a productive Rad51 presynaptic filament on RPA-coated ssDNA. Sgs1 binds RPA via an acidic region just upstream of the helicase core (HEGNAUER \textit{et al.} 2012). However, the biological significance of this interaction has remained unclear. We propose that the acidic region in the N-terminus of Sgs1 to which RPA binds serves as a DNA mimic, and that via this DNA mimic Sgs1 can compete with the ssDNA overhang for RPA binding, thereby freeing up ssDNA locally for Rad51 and stimulating filament initiation (Figure 7A). This model is supported by the overlap between the distinct phenotype of the \textit{sgs1-FD} mutant and phenotypes of mutations in the acidic region of Sgs1, including suppression of \textit{top3Δ} slow growth, wildtype-level resistance to HU and MMS, lack of a hyper-recombination phenotype, and lack of synthetic lethal interactions characteristic of the \textit{sgs1Δ} mutation (BERNSTEIN \textit{et al.} 2009; BERNSTEIN \textit{et al.} 2013). Such a role for Sgs1 is also reminiscent of the function of \textit{E. coli} RecBCD not only in resection, but also in assembling the RecA filament (ANDERSON and KOWALCZYKOWSKI 1997). There is also in vitro evidence that
the BLM-hRad51 interaction may play a role in loading hRad51 onto ends resected by BLM and Exo1 (Nimonkar et al. 2008). Moreover, the ability of BRCA2 to load Rad51 onto ssDNA in vitro was recently shown to be aided by interaction with a protein, DSS1, that appears to act as a DNA mimic and targets RPA on ssDNA (Zhao et al. 2015).

We also observed a stimulatory effect of the Sgs1-Rad51 interaction on the formation of the direct-repeat mediated, large deletions characteristic of pol32Δ cells. Unlike in DSB resection, however, Sgs1 had the opposite effect of Exo1: the Sgs1-Rad51 interaction promoted the deletions, and Exo1 suppressed them, both to approximately the same extent. The large deletions in pol32Δ cells most likely form during inefficient replisome progression, which makes the nascent DNA strands prone to dissociation, followed by misannealing at a repeated downstream sequence, thus deleting the sequence between the repeats. We propose a model whereby Exo1 prevents large deletions through its ability to degrade the nascent lagging DNA strand at stalled forks (Engels et al. 2011) and the Sgs1-Rad51 interaction, in contrast, promotes annealing of dissociated nascent strands with downstream repeated sequences (Figure 7A).

Finally, our study also provides new insight into the repair of DSBs in cells where long-range resection by Sgs1/Dna2 and Exo1 is disrupted. It is thought that for HR, the ends need to be resected extensively by Sgs1/Dna2 or Exo1 before a productive Rad51 filament can form and initiate a homology search. Hence, sgs1Δ exo1Δ mutants should not be able to rely on HR as a major pathway for DNA lesion repair. It was therefore surprising that sgs1Δ exo1Δ cells depend on RAD52 for their survival. That the fitness of these cells was more dependent on Rad59 than Rad51 suggests that the minimally resected DSB ends in sgs1Δ exo1Δ cells are mainly repaired by Rad59/Rad52-dependent
HR. This is consistent with a preference of Rad59 for the repair of short substrates, including by Rad51-independent BIR (Sugawara et al. 2000; Ira and Haber 2002; Pannunzio et al. 2008). Interestingly, we also found that Rad59 suppressed genome rearrangements in sgs1Δ exo1Δ mutants whereas Rad51 increased them, suggesting that both Rad51 and Rad59 can act on minimally resected ends, but with Rad59 leading to proper repair, whereas Rad51 is mutagenic. That the genetic interactions between SGS1, RAD51 and RAD59 were the same in the presence or absence of EXO1 – that is, Rad59 suppressed GCRs in the absence of Sgs1 whereas Rad51 generated them – further indicates that sgs1Δ and sgs1Δ exo1Δ cells simply differ in the abundance of the lesions, but the lesions are of the same type and accessed in the same manner by Rad59 and Rad51 whether Exo1 is present or not.

In addition to Rad51, Sgs1 interacts with numerous other DNA repair factors, including Top2, Top3, RPA, Mre11, Rad16, and Mlh1, and the checkpoint kinase Rad53. However, determining the significance of these interactions for Sgs1 function has remained challenging due to the lack of point mutations that disrupt individual interactions. Identifying the binding sites on Sgs1 for these other interacting partners will allow to further dissect the well-characterized, but pleiotropic, effect of an SGS1 deletion on DNA break repair and provide a more precise understanding of the specific roles of Sgs1 in promoting genome integrity.

ACKNOWLEDGEMENTS

We thank Sue Jinks-Robertson (Duke University) for plasmids for the gap repair assay and Richard Kolodner (UC San Diego) for yeast strains. This work was supported by
LITERATURE CITED

AMIN, A. D., A. B. CHAIX, R. P. MASON, R. M. BADGE and R. H. BORTS, 2010 The roles of the Saccharomyces cerevisiae RecQ helicase SGS1 in meiotic genome surveillance. PLoS One 5: e15380.

ANDERSON, D. G., and S. C. KOWALCZYKOWSKI, 1997 The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. Cell 90: 77-86.

BARLOW, J. H., M. LISBY and R. ROTHSTEIN, 2008 Differential regulation of the cellular response to DNA double-strand breaks in G1. Mol Cell 30: 73-85.

BERNSTEIN, K. A., E. P. MIMITOU, M. J. MIHALEVIC, H. CHEN, I. SUNJEVERIC et al., 2013 Resection activity of the Sgs1 helicase alters the affinity of DNA ends for homologous recombination proteins in Saccharomyces cerevisiae. Genetics 195: 1241-1251.

BERNSTEIN, K. A., E. SHOR, I. SUNJEVERIC, M. FUMASONI, R. C. BURGESS et al., 2009 Sgs1 function in the repair of DNA replication intermediates is separable from its role in homologous recombinational repair. Embo J 28: 915-925.

BURGERS, P. M., and K. J. GEREK, 1998 Structure and processivity of two forms of Saccharomyces cerevisiae DNA polymerase delta. J Biol Chem 273: 19756-19762.

CANNANO, E., and P. CEJKA, 2014 Sac2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. Nature 514: 122-125.

CANNANO, E., P. CEJKA and S. C. KOWALCZYKOWSKI, 2013 Relationship of DNA degradation by Saccharomyces cerevisiae exonuclease 1 and its stimulation by RPA and Mre11-Rad50-Xrs2 to DNA end resection. Proc Natl Acad Sci U S A 110: E1661-1668.

CEJKA, P., E. CANNANO, P. POLACZEK, T. MASUDA-SASA, S. POKHAREL et al., 2010a DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. Nature 467: 112-116.

CEJKA, P., J. L. PLANK, C. Z. BACHRATI, I. D. HICKSON and S. C. KOWALCZYKOWSKI, 2010b Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. Nat Struct Mol Biol 17: 1377-1382.

CEJKA, P., J. L. PLANK, C. C. DOMBROWSKI and S. C. KOWALCZYKOWSKI, 2012 Decatenation of DNA by the S. cerevisiae Sgs1-Top3-Rmi1 and RPA complex: a mechanism for disentangling chromosomes. Mol Cell 47: 886-896.
CHANG, M., M. BELLAQUI, C. ZHANG, R. DESAI, P. MOROZOV et al., 2005 RMI1/NCE4, a suppressor of genome instability, encodes a member of the RecQ helicase/Topo III complex. Embo J 24: 2024-2033.
CHEN, H., R. A. DONNIANNI, N. HANSA, S. K. DENG, J. OH et al., 2015 Sae2 promotes DNA damage resistance by removing the Mre11-Rad50-Xrs2 complex from DNA and attenuating Rad53 signaling. Proc Natl Acad Sci U S A 112: E1880-1887.
CHIOLO, I., W. CAROSENUTO, G. MAFFIOLETTI, J. H. PETRINI, M. FOHANI et al., 2005 Srs2 and Sgs1 DNA helicases associate with Mre11 in different subcomplexes following checkpoint activation and CDK1-mediated Srs2 phosphorylation. Mol Cell Biol 25: 5738-5751.
CLERICI, M., D. MANTIERO, G. LUCCHINI and M. P. LONGHESE, 2006 The Saccharomyces cerevisiae Sae2 protein negatively regulates DNA damage checkpoint signalling. EMBO Rep 7: 212-218.
DHERIN, C., E. GUENEAU, M. FRANCIN, M. NUNEZ, S. MIRON et al., 2009 Characterization of a highly conserved Mlh1 required for exonuclease I-dependent mismatch repair. Mol Cell Biol 29: 907-918.
DOERFLER, L., L. HARRIS, E. VIEBRANZ and K. H. SCHMIDT, 2011 Differential genetic interactions between Sgs1, DNA-damage checkpoint components and DNA repair factors in the maintenance of chromosome stability. Genome Integr 2: 8.
DOERFLER, L., and K. H. SCHMIDT, 2014 Exo1 phosphorylation status controls the hydroxyurea sensitivity of cells lacking the Pol32 subunit of DNA polymerases delta and zeta. DNA Repair (Amst) 24: 26-36.
ENGELS, K., M. GIANNATTASIO, M. MUZI-FALCONI, M. LOPES and S. FERRARI, 2011 14-3-3 Proteins regulate exonuclease 1-dependent processing of stalled replication forks. PLoS Genet 7: e1001367.
FASCHING, C. L., P. CJEKA, S. C. KOWALCZYKOWSKI and W. D. HEYER, 2015 Top3-Rmi1 dissolve Rad51-mediated D loops by a topoisomerase-based mechanism. Mol Cell 57: 595-606.
FERRARI, M., D. DIBITETTO, G. DE GREGORIO, V. V. EAPEN, C. C. RAWAL et al., 2015 Functional interplay between the 53BP1-ortholog Rad9 and the Mre11 complex regulates resection, end-tethering and repair of a double-strand break. PLoS Genet 11: e1004928.
FRANK-VAILLANT, M., and S. MARCAND, 2001 NHEJ regulation by mating type is exercised through a novel protein, Lif2p, essential to the ligase IV pathway. Genes Dev 15: 3005-3012.
FRICKE, W. M., and S. J. BRILL, 2003 Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev 17: 1768-1778.
FRICKE, W. M., V. KALIRAMAN and S. J. BRILL, 2001 Mapping the DNA topoisomerase III binding domain of the Sgs1 DNA helicase. J Biol Chem 276: 8848-8855.
GANGLOFF, S., J. P. MCDONALD, C. BENDIXEN, L. ARTHUR and R. ROTHSTEIN, 1994 The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. Mol Cell Biol 14: 8391-8398.
GANGLOFF, S., C. SOUSTELLE and F. FABRE, 2000 Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. Nat Genet 25: 192-194.
Gietz, R. D., and R. A. Woods, 2006 Yeast transformation by the LiAc/SS Carrier DNA/PEG method. Methods Mol Biol 313: 107-120.

Gobbini, E., M. Villa, M. Gnugnoli, L. Menin, M. Clerici et al., 2015 Sae2 Function at DNA Double-Strand Breaks Is Bypassed by Dampening Tel1 or Rad53 Activity. PLoS Genet 11: e1005685.

Gravel, S., J. R. Chapman, C. Magill and S. P. Jackson, 2008 DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes Dev 22: 2767-2772.

Hegnauer, A. M., N. Hustedt, K. Shimada, B. L. Pike, M. Vogel et al., 2012 An N-terminal acidic region of Sgs1 interacts with Rpa70 and recruits Rad53 kinase to stalled forks. Embo J 31: 3768-3783.

Huang, M. E., A. G. Rio, M. D. Galibert and F. Galibert, 2002 Pol32, a subunit of Saccharomyces cerevisiae DNA polymerase delta, suppresses genomic deletions and is involved in the mutagenic bypass pathway. Genetics 160: 1409-1422.

Huertas, P., F. Cortes-Ledesma, A. A. Sartori, A. Aguilera and S. P. Jackson, 2008 CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature 455: 689-692.

Ira, G., and J. E. Haber, 2002 Characterization of RAD51-independent break-induced replication that acts preferentially with short homologous sequences. Mol Cell Biol 22: 6384-6392.

Ira, G., A. Pellicioli, A. Baliija, X. Wang, S. Fiorani et al., 2004 DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature 431: 1011-1017.

Ivesa, A. S., J. Q. Zhou, V. P. Schulz, E. K. Monson and V. A. Zakian, 2002 Saccharomyces Rrm3p, a 5' to 3' DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. Genes Dev 16: 1383-1396.

Johansson, E., P. Garg and P. M. Burgers, 2004 The Pol32 subunit of DNA polymerase delta contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. J Biol Chem 279: 1907-1915.

Kennedy, J. A., G. W. Daughdrill and K. H. Schmidt, 2013 A transient alpha-helical molecular recognition element in the disordered N-terminus of the Sgs1 helicase is critical for chromosome stability and binding of Top3/Rmi1. Nucleic Acids Res 41: 10215-10227.

Krejci, L., S. Van Komen, Y. Li, J. Villemain, M. S. Reddy et al., 2003 DNA helicase Srs2 disrupts the Rad51 presynaptic filament. Nature 423: 305-309.

Lea, D. E., and C. A. Coulson, 1949 The distribution of the numbers of mutants in bacterial populations. Journal of Genetics 49: 264-285.

Lee, S. K., R. E. Johnson, S. L. Yu, L. Prakash and S. Prakash, 1999 Requirement of yeast SGS1 and SRS2 genes for replication and transcription. Science 286: 2339-2342.

Levikova, M., C. Pinto and P. Cejka, 2017 The motor activity of DNA2 functions as an ssDNA translocase to promote DNA end resection. Genes Dev 31: 493-502.

Li, X. C., and B. K. Tye, 2011 Ploidy dictates repair pathway choice under DNA replication stress. Genetics 187: 1031-1040.
LISBY, M., J. H. BARLOW, R. C. BURGESS and R. ROTHSTEIN, 2004 Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118: 699-713.

LIU, J., L. RENAUT, X. VEAUTE, F. FABRE, H. STAILBERG et al., 2011 Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. Nature 479: 245-248.

MIMITOU, E. P., and L. S. SYMINGTON, 2008 Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455: 770-774.

MIMITOU, E. P., and L. S. SYMINGTON, 2010 Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J 29: 3358-3369.

MIRZAEI, H., and K. SCHMIDT, 2012 Non-Bloom-syndrome-associated partial and total loss-of-function variants of BLM helicase. Proc Natl Acad Sci U S A.

MIRZAEI, H., S. SYED, J. KENNEDY and K. H. SCHMIDT, 2011 Sgs1 truncations induce genome rearrangements but suppress detrimental effects of BLM overexpression in Saccharomyces cerevisiae. J Mol Biol 405: 877-891.

MITCHEL, K., H. ZHANG, C. WELZ-VOEGELE and S. JINKS-ROBERTSON, 2010 Molecular structures of crossover and noncrossover intermediates during gap repair in yeast: implications for recombination. Mol Cell 38: 211-222.

MULLEN, J. R., V. KALIRAMAN and S. J. BRILL, 2000 Bipartite structure of the SGS1 DNA helicase in Saccharomyces cerevisiae. Genetics 154: 1101-1114.

MULLEN, J. R., V. KALIRAMAN, S. S. IBRAHIM and S. J. BRILL, 2001 Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in Saccharomyces cerevisiae. Genetics 157: 103-118.

MULLEN, J. R., F. S. NALLASETH, Y. Q. LAN, C. E. SLAGLE and S. J. BRILL, 2005 Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. Mol Cell Biol 25: 4476-4487.

MYUNG, K., A. DATTA, C. CHEN and R. D. KOLODNER, 2001 SGS1, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination. Nat Genet 27: 113-116.

NAIR, K. R., 1940 Table of confidence intervals for the median in samples from any continuous population. Sankhya: 551-558.

NEW, J. H., T. SUGIYAMA, E. ZAITSEVA and S. C. KOWALCZYKOWSKI, 1998 Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature 391: 407-410.

NICOLETTE, M. L., K. LEE, Z. GUO, M. RANI, J. M. CHOW et al., 2010 Mre11-Rad50-Xrs2 and Sae2 promote 5' strand resection of DNA double-strand breaks. Nat Struct Mol Biol 17: 1478-1485.

NIMONKAR, A. V., A. Z. OZSOY, J. GENSCHEL, P. MODRICH and S. C. KOWALCZYKOWSKI, 2008 Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. Proc Natl Acad Sci U S A 105: 16906-16911.

NIU, H., W. H. CHUNG, Z. ZHU, Y. KWON, W. ZHAO et al., 2010 Mechanism of the ATP-dependent DNA end-resection machinery from Saccharomyces cerevisiae. Nature 467: 108-111.
OOI, S. L., D. D. SHOEMAKER and J. D. BOEKE, 2003 DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. Nat Genet 35: 277-286.

PAN, X., P. YE, D. S. YUAN, X. WANG, J. S. BADER et al., 2006 A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell 124: 1069-1081.

PANNUNZIO, N. R., G. M. MANTHEY and A. M. BAILIS, 2008 RAD59 is required for efficient repair of simultaneous double-strand breaks resulting in translocations in Saccharomyces cerevisiae. DNA Repair (Amst) 7: 788-800.

PEDRAZZI, G., C. PERRERA, H. BLASER, P. KUSTER, G. MARRA et al., 2001 Direct association of Bloom's syndrome gene product with the human mismatch repair protein MLH1. Nucleic Acids Res 29: 4378-4386.

PUDDU, F., T. OELSCHLAEGEL, I. GUERINI, N. J. GEISLER, H. NIU et al., 2015 Synthetic viability genomic screening defines Sae2 function in DNA repair. EMBO J 34: 1509-1522.

REENAN, R. A., and R. D. KOLODNER, 1992 Characterization of insertion mutations in the Saccharomyces cerevisiae MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions. Genetics 132: 975-985.

SAFFI, J., H. FELDMANN, E. L. WINNACKER and J. A. HENRIQUES, 2001 Interaction of the yeast Pso5/Rad16 and Sgs1 proteins: influences on DNA repair and aging. Mutat Res 486: 195-206.

SCHMIDT, K. H., and R. D. KOLODNER, 2004 Requirement of Rrm3 helicase for repair of spontaneous DNA lesions in cells lacking Srs2 or Sgs1 helicase. Mol Cell Biol 24: 3213-3226.

SCHMIDT, K. H., E. VIEBRANZ, L. DOERFLER, C. LESTER and A. RUBENSTEIN, 2010a Formation of complex and unstable chromosomal translocations in yeast. PLoS One 5: e12007.

SCHMIDT, K. H., E. B. VIEBRANZ, L. B. HARRIS, H. MIRZAEI-SOUDERJANI, S. SYED et al., 2010b Defects in DNA lesion bypass lead to spontaneous chromosomal rearrangements and increased cell death. Eukaryot Cell 9: 315-324.

SCHMIDT, K. H., J. WU and R. D. KOLODNER, 2006 Control of Translocations between Highly Diverged Genes by Sgs1, the Saccharomyces cerevisiae Homolog of the Bloom's Syndrome Protein. Mol Cell Biol 26: 5406-5420.

SHIM, E. Y., W. H. CHUNG, M. L. NICOLETTE, Y. ZHANG, M. DAVIS et al., 2010 Saccharomyces cerevisiae Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. EMBO J 29: 3370-3380.

SHOR, E., S. GANGLOFF, M. WAGNER, J. WEINSTEIN, G. PRICE et al., 2002 Mutations in homologous recombination genes rescue top3 slow growth in Saccharomyces cerevisiae. Genetics 162: 647-662.

SIGNON, L., and M. N. SIMON, 2014 The analysis of S. cerevisiae cells deleted for mitotic cyclin Clb2 reveals a novel requirement of Sgs1 DNA helicase and Exonuclease 1 when replication forks break in the presence of alkylation damage. Mutat Res 769: 80-92.

SINCLAIR, D. A., K. MILLS and L. GUARENTE, 1997 Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. Science 277: 1313-1316.
SONG, B., and P. SUNG, 2000 Functional interactions among yeast Rad51 recombinase, Rad52 mediator, and replication protein A in DNA strand exchange. J Biol Chem 275: 15895-15904.

SPELL, R. M., and S. JINKS-ROBERTSON, 2004 Examination of the Roles of Sgs1 and Srs2 Helicases in the Enforcement of Recombination Fidelity in Saccharomyces cerevisiae. Genetics 168: 1855-1865.

SUGAWARA, N., G. IRA and J. E. HABEL, 2000 DNA length dependence of the single-strand annealing pathway and the role of Saccharomyces cerevisiae RAD59 in double-strand break repair. Mol Cell Biol 20: 5300-5309.

SYED, S., C. DESLER, L. J. RASMUSSEN and K. H. SCHMIDT, 2016 A Novel Rrm3 Function in Restricting DNA Replication via an Orc5-Binding Domain Is Genetically Separable from Rrm3 Function as an ATPase/Helicase in Facilitating Fork Progression. PLoS Genet 12: e1006451.

TONG, A. H., M. EVANGELISTA, A. B. PARSONS, H. XU, G. D. BADER et al., 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294: 2364-2368.

TORRES, J. Z., S. L. SCHNakenberg and V. A. ZAKIAN, 2004 Saccharomyces cerevisiae Rrm3p DNA helicase promotes genome integrity by preventing replication fork stalling: viability of rrm3 cells requires the intra-S-phase checkpoint and fork restart activities. Mol Cell Biol 24: 3198-3212.

TRUJILLO, K. M., D. H. ROH, L. CHEN, S. VAN KOMEN, A. TOMKINSON et al., 2003 Yeast xrs2 binds DNA and helps target rad50 and mre11 to DNA ends. J Biol Chem 278: 48957-48964.

UI, A., M. SEKI, H. OGIWARA, R. ONODERA, S. FUKUSHIGE et al., 2005 The ability of Sgs1 to interact with DNA topoisomerase III is essential for damage-induced recombination. DNA Repair (Amst) 4: 191-201.

USUI, T., H. OGAWA and J. H. PETRINI, 2001 A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol Cell 7: 1255-1266.

VEAUTE, X., J. JEUSSET, C. SOUSTELLE, S. C. KOWALCZYKOWSKI, E. LE CAM et al., 2003 The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature 423: 309-312.

WANG, T. F., and W. M. KUNG, 2002 Supercomplex formation between Mlh1-Mlh3 and Sgs1-Top3 heterocomplexes in meiotic yeast cells. Biochem Biophys Res Commun 296: 949-953.

WATT, P. M., E. J. LOUIS, R. H. BORTS and I. D. HICKSON, 1995 Sgs1: a eukaryotic homolog of E. coli RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell 81: 253-260.

WELZ-VOEGELE, C., and S. JINKS-ROBERTSON, 2008 Sequence divergence impedes crossover more than noncrossover events during mitotic gap repair in yeast. Genetics 179: 1251-1262.

WU, L., S. L. DAVIES, N. C. LEVITT and I. D. HICKSON, 2001 Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51. J Biol Chem 276: 19375-19381.

WU, L., and I. D. HICKSON, 2003 The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 426: 870-874.
ZHao, W., S. Vaithiyalingam, J. San Filippo, D. G. Maranon, J. Jimenez-Sainz et al., 2015 Promotion of BRCA2-Dependent Homologous Recombination by DSS1 via RPA Targeting and DNA Mimicry. Mol Cell 59: 176-187.

Zhu, Z., W. H. Chung, E. Y. Shim, S. E. Lee and G. Ira, 2008 Sgs1 helicase and two nucleasees Dna2 and Exo1 resect DNA double-strand break ends. Cell 134: 981-994.
TABLE 1. Effect of the sgs1-FD mutation on the rate of accumulation of gross-chromosomal rearrangements (GCRs).

| Relevant genotype | GCR rate \( (\text{Can}^\text{' 5-FOA} \times 10^{-16}) \) | 95% CI \( (\text{Can}^\text{' 5-FOA} \times 10^{-16}) \) |
|------------------|---------------------------------|------------------|
| wildtype         | 1.1                             | <1 – 6.2         |
| sgs1             | 71                              | 53-104           |
| sgs1-FA          | <9                              | <7-9             |
| sgs1-FD          | <7                              | <6-8             |
| exo1             | 14                              | 7-28             |
| sgs1 exo1        | 40500                           | 31000-49900      |
| sgs1-FD exo1     | 83                              | 49-124           |
| sae2             | 12                              | <7-18            |
| sgs1-FD sae2     | 306                             | (124-424)        |
| mre11            | 2200                            | n.d.             |
| mre11 sgs1-FD    | 2030                            | 1170-2480        |
| top3             | 27                              | 17-96            |
| top3 sgs1-FD     | 12                              | 9-36             |
| rad24            | 23                              | 9-37             |
| sgs1 rad24       | 136                             | 117-216          |
| sgs1-FD rad24    | 26                              | 10-69            |
| pol32            | 20                              | 15-26            |
| sgs1 pol32       | 25                              | <24-105          |
| sgs1-FD pol32    | <8                              | <7-19            |
| rrm3             | 14                              | 5-28             |
| sgs1 rrm3        | 656                             | 311-1290         |
| sgs1-FD rrm3     | <6                              | <5-8             |
| srs2             | 0.6                             | <2-11            |
| sgs1-FD srs2     | 8                               | <7-11            |

\(^{a}\) Gross-chromosomal rearrangement (GCR) rates for \textit{mre11} and \textit{top3} mutants are from \textsc{Myung et al.} 2001; for \textit{sgs1} \textit{rrm3} from \textsc{Schmidt and Kolodner} 2006b; for \textit{sgs1} \textit{exo1} and \textit{exo1} from \textsc{Doerfler and Schmidt} 2014; and for \textit{srs2} from \textsc{Schmidt et al.} 2010b.

\(^{b}\) 95% confidence intervals (CI) were calculated according to \textsc{Nair et al.} 1940.
Table 2. Differential effects of RAD51 and RAD59 deletions on gross-chromosomal rearrangement (GCR) formation in sgs1 and sgs1 exo1 mutants

| Genotype       | GCR Rate\(^a\) (Can’ 5-FOA’ x 10\(^{-10}\)) | 95% CI\(^b\) (Can’ 5-FOA’ x 10\(^{-10}\)) |
|----------------|-------------------------------------------|------------------------------------------|
| wildtype       | 1.1                                       | < 1 – 6.2                                 |
| sgs1           | 71                                        | 53-104                                   |
| exo1           | 14                                        | 7-28                                     |
| rad51          | < 8                                       | < 7-15                                   |
| rad59          | 24                                        | 13-50                                    |
| rad52          | 138                                       | 16-267                                   |
| sgs1 exo1      | 40500                                     | 31000-49900                              |
| sgs1 rad51     | 14                                        | 12-24                                    |
| sgs1 rad59     | 126                                       | 107-300                                  |
| sgs1 rad52     | 308                                       | 140-452                                  |
| sgs1 rad51 exo1| 13100                                     | 8520-21900                               |
| sgs1 rad59 exo1| 94900                                     | 67400-185000                             |

\(^a\) Gross-chromosomal rearrangement (GCR) rate for sgs1 rad59 is from DOERFLER et al. 2011.

\(^b\) 95% confidence intervals (CI) were calculated according to NAIR et al. 1940.
FIGURE LEGENDS

Figure 1. Rad51 interacts with Sgs1 downstream of the winged-helix domain. (A) Domain structure of Sgs1. The helicase core of Sgs1 consists of the ATPase domain, which is formed by two RecA-like lobes, and the RecQ-C terminal (RQC) domain, which consists of a zinc-binding (Zinc-BD) and a winged-helix (WH) domain. The helicase-and RNaseD C-terminal (HRDC) domain is separated from the helicase core by a proline/glycine-rich loop. The 645-residue unstructured N-terminal tail is omitted. (B) Pulldown of Rad51-V5-3XVSV with GST-tagged Sgs1 fragments and sgs1-FD and sgs1-FA mutants. (C) Expression levels of the sgs1-FD allele from the chromosomal SGS1 promoter do not differ from the wildtype SGS1 allele. Whole cell extracts were prepared by trichloroacetic acid extraction from an equal number of cells expressing myc-epitope-tagged sgs1-FD or wildtype Sgs1 as previously described (KENNEDY et al. 2013) and analyzed by Western blot using a monoclonal (9E10) c-myc antibody (SCBT). (D) Unlike an SGS1 deletion, the sgs1-FD allele does not cause hypersensitivity to MMS or HU in haploid cells. Homozygosity for the sgs1-FD mutation causes mild sensitivity to HU and MMS in diploid cells.

Figure 2. Effect of the sgs1-FD mutation on fitness, DNA-damage sensitivity and genome stability of sae2Δ, mre11Δ and yku70Δ mutants. (A) sgs1-FD causes a severe growth defect in the sae2Δ mutant, but not the mre11Δ mutant. Deletion of MRE11 suppresses the growth defect of sgs1-FD sae2Δ. (B) sgs1-FD increases the HU/MMS sensitivity of the sae2Δ mutant, but not the mre11Δ mutant. (C) Unlike mre11Δ, yku70Δ
does not suppress the growth defect and HU/MMS hypersensitivity of the *sgs1-FD sae2Δ* mutant. (D) *sgs1-FD* causes synergistic GCR rate increases in *sae2Δ* and *exo1Δ* mutants, but has no effect on GCR accumulation in the *mre11Δ* mutant. Median GCR rates are shown with 95% confidence intervals (see also Table 1).

**Figure 3. Effect of the sgs1-FD mutation on fitness and DNA-damage sensitivity of *exo1Δ*, *srs2Δ*, *rrm3Δ* and *top3Δ* mutants.** (A) *sgs1-FD* sensitizes the *exo1Δ* mutant to MMS and, to a lesser extent, HU. (B) *sgs1-FD* suppresses HU/MMS sensitivity of the *srs2Δ* mutant and increases HU/MMS sensitivity of the *rrm3Δ* mutant. (C) Unlike deletion of *SGS1*, *sgs1-FD* does not cause a GCR rate increase in the *rrm3Δ* mutant. *sgs1-FD* does not affect GCR formation in the *srs2Δ* mutant (see also Table 1). Median GCR rates are shown with 95% confidence intervals.

**Figure 4. Disrupting Sgs1-Rad51 interaction suppresses the severe growth defect of the top3 mutant, but has no effect on crossover/noncrossover formation.**

(A) *sgs1-FD* suppresses *top3Δ* slow growth nearly as effectively as a deletion of *SGS1* or helicase-dead *sgs1-HD*. (B) Improved growth of *sgs1-FD top3Δ* in the presence of HU and MMS correlates with partial suppression of the *top3Δ* fitness defect by *sgs1-FD*. (C) In contrast to an *SGS1* deletion, the *sgs1-FD* mutation does not have a major effect on the ratio between crossover/noncrossover outcomes in gap repair. Over 140 His⁺ transformants each from two independent *can1::his3-0,Δ3’* isolates of the wildtype, *sgs1-FD* and *sgs1Δ* strains were analyzed.
Figure 5. Effect of sgs1-FD on DNA-damage sensitivity and large deletion formation in the absence of Pol32. (A) Suppression of hydroxyurea sensitivity of the pol32Δ mutant by sgs1-FD. In contrast, SGS1 deletion or helicase-defective sgs1-HD increase DNA-damage sensitivity of pol32Δ cells. (B) Accumulation of large deletions (> 3 bp) in CAN1, characteristic of cells lacking the Pol32 subunit of polymerase δ, is suppressed by sgs1-FD or by deletion of SGS1, but stimulated by deletion of EXO1. (C) Whereas exo1Δ and sgs1-FD suppress HU sensitivity of the pol32Δ mutant, combination of both exo1Δ and sgs1-FD mutations increases DNA damage sensitivity of the pol32Δ mutant. (D) Deletion of RAD51 in the pol32Δ mutant causes severe hypersensitivity to HU and MMS.

Figure 6. Effect of RAD52, RAD51 and RAD59 deletions on viability and genome stability of the sgs1Δ exo1Δ mutant. (A) As shown by tetrad dissections, deletion of RAD52 in the sgs1Δ exo1Δ mutant is lethal, and deletion of RAD59 causes a severe growth defect. In contrast, deletion of RAD51 causes only a mild growth defect. Triple mutant spores are indicated by a white circle. (B) Deletion of RAD51 suppresses gross-chromosomal rearrangements (GCRs) in the sgs1Δ mutant whereas deletion of RAD59 stimulates GCR formation in the sgs1Δ mutant. (C) Deletion of EXO1 increases GCR formation in the sgs1Δ mutant ~700-fold, but does not affect the genetic interactions of sgs1Δ with rad51Δ and rad59Δ. Median GCR rates are shown with 95% confidence intervals.

Figure 7. Model for a stimulatory role of the Sgs1-Rad51 interaction in homology-dependent repair of spontaneous DNA lesions. (A) Replication stress, exogenous DNA
damage, or disruption of factors with roles in replisome progression (e.g., \textit{rrm3}\Delta, \textit{pol32}\Delta) can impair replication forks and give rise to mutations (left) and DSBs (right).

\textit{Right panel:} Unprocessed DNA breaks can be bound by Ku and MRX. Nuclease activity of MRX/Sae2 trims the ends, which are then extensively resected by Exo1 and Sgs1/Dna2. Bound Ku and MRX inhibit long-range resection by Exo1 and Sgs1/Dna2, respectively. As the \textit{sgs1-FD} mutant does not benefit from \textit{YKU70} deletion, suggesting it does not have a significant resection defect, we propose that the Sgs1-Rad51 interaction could instead stimulate HR by linking Sgs1’s role in long-range resection to Rad51 filament formation. Specifically, the acidic regions (AR) in the unstructured N-terminal tail of Sgs1, through their capacity to bind RPA, could act as a DNA mimic, allowing Sgs1 to compete with ssDNA for RPA binding, thereby facilitating deposition of Sgs1-bound Rad51 onto ssDNA during resection. \textit{Note:} Ku, MRX, Exo1 and Sgs1/Dna2 can act on the same DSB end; two ends are shown to separate their activities for clarity only.

\textit{Left panel:} In the absence of Pol32, cells are known to accumulate large deletions of sequences flanked by direct short repeats. We propose a model whereby Sgs1, through its interaction with Rad51, stimulates the formation of these deletions (Figure 5B) by mediating misannealing of the nascent strands with downstream repeated sequences, whereas the 5’-3’ exonuclease Exo1 reduces deletion formation (Figure 5B) by degrading nascent DNA on the lagging strand from its accessible 5’ end. \textbf{(B)} Summary of differential genetic interactions of the \textit{sgs1-FD} allele and the \textit{SGS1} deletion with mutations in DNA recombination and replication factors (n.a., not applicable; n.d., not determined).
A

N-terminal acidic regions (AR) of Sgs1 may act as a DNA mimic that competes with ssDNA for RPA binding during resection, thereby facilitating loading of Rad51.

B

Summary of the genetic interactions of the *sgs1-FD* mutation investigated in this study

|          | *sgs1Δ*   | *sgs1-FD*     | sgs1-FD sae2Δ |
|----------|-----------|---------------|---------------|
| *yku70A* | none      | none          | none          |
| *mre11A* | negative (lethal) | none          | positive (rescue) |
| *sae2Δ*  | negative (lethal) | negative (sick, HU/MM) | n.a.          |
| *srs2Δ*  | negative (lethal) | positive (rescue) | n.d.          |
| *exo1Δ*  | negative (sick, HU/MM) | negative (HU/MM) | n.d.          |
| *pol32Δ* | negative (sick, HU/MM) | positive (rescue) | n.d.          |
| *rrm3Δ*  | negative (lethal) | negative (HU/MM) | n.d.          |
| *top3Δ*  | positive (rescue) | positive (rescue) | n.d.          |