The Short Life Span of *Saccharomyces cerevisiae sgs1* and *srs2* Mutants Is a Composite of Normal Aging Processes and Mitotic Arrest Due to Defective Recombination

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ABSTRACT

Evidence from many organisms indicates that the conserved RecQ helicases function in the maintenance of genomic stability. Mutation of *SGS1* and *WRN*, which encode RecQ homologues in budding yeast and humans, respectively, results in phenotypes characteristic of premature aging. Mutation of *SRS2*, another DNA helicase, causes synthetic slow growth in an *sgs1* background. In this work, we demonstrate that *srs2* mutants have a shortened life span similar to *sgs1* mutants. Further dissection of the *sgs1* and *srs2* survival curves reveals two distinct phenomena. A majority of *sgs1* and *srs2* cells stops dividing stochastically as large-budded cells. This mitotic cell cycle arrest is age independent and requires the *RAD9*-dependent DNA damage checkpoint. Late-generation *sgs1* and *srs2* cells senesce due to apparent premature aging, most likely involving the accumulation of extrachromosomal rDNA circles. Double *sgs1 srs2* mutants are viable but have a high stochastic rate of terminal G2/M arrest. This arrest can be suppressed by mutations in *RAD51*, *RAD52*, and *RAD57*, suggesting that the cell cycle defect in *sgs1 srs2* mutants results from inappropriate homologous recombination. Finally, mutation of *RAD1* or *RAD50* exacerbates the growth defect of *sgs1 srs2* cells, indicating that *sgs1 srs2* mutants may utilize single-strand annealing as an alternative repair pathway.

THE use of model organisms has greatly contributed to the identification of some of the underlying causes of aging. In particular, the budding yeast *Saccharomyces cerevisiae* has proven to be an effective system for studying the mechanisms of cellular senescence. Many genes that affect yeast life span have been identified (reviewed in Jazwinski 1999). Of particular interest is *SGS1*, a member of the RecQ family of DNA helicases. Mutation of *SGS1* results in a 60% reduction in mean life span (Sinclair *et al.* 1997). Furthermore, many phenotypes of yeast aging occur early in *sgs1* mutants, including sterility, fragmentation of the nucleolus, and movement of the Sir proteins to the nucleolus. In humans, mutation of the RecQ homologues WRN, BLM, or RECQL4 results in genetic disorders characterized by increased genomic instability, a predisposition to certain types of cancers, and in the case of WRN, hallmarks of premature aging (reviewed in Karow *et al.* 2000). Therefore, a clear understanding of the cellular roles performed by Sgs1p in yeast could provide clues to the molecular basis of these diseases.

Sgs1p has demonstrated 3′-5′ DNA helicase activity (Bennett *et al.* 1998). It binds preferentially to branched DNA substrates, including duplex structures with 3′ single-stranded overhangs and DNA junctions with multiple branches (Bennett *et al.* 1998). *In vitro*, it is more efficient at unwinding G-G paired DNA compared to duplex DNA (Sun *et al.* 1999). Yeast cells lacking Sgs1p have a mitotic hyperrecombination phenotype, with increases in both intra- and interchromosomal homologous recombination (Watt *et al.* 1996). This increased recombination occurs both at the highly repetitive ribosomal DNA (rDNA) locus and at other sites in the genome (Gangloff *et al.* 1994; Watt *et al.* 1996). Because the rDNA is G-rich, one proposed function for Sgs1p is the prevention of recombination within the rDNA, possibly by the resolution of G-G paired DNA duplexes (Sun *et al.* 1999).

Intrachromosomal recombination within the rDNA can result in the formation of extrachromosomal ribosomal DNA circles (ERCs), which have been shown to be a cause of aging in yeast (Sinclair and Guarente 1997). Therefore, it was proposed that Sgs1p promotes longevity in yeast by decreasing the rate of recombination and the formation of ERCs. However, recently published data demonstrate that *sgs1* mutants do not accumulate ERCs more rapidly than wild-type cells (Heo *et al.* 1999), suggesting additional mechanisms may promote premature aging in *sgs1* cells.

Further insight into the role(s) of Sgs1p comes from its interactions with the topoisomerases. Topoisomerases are able to relieve torsional stress in DNA double

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helices and separate intertwined DNA molecules (Wang 1996). Sgs1p interacts with both Top2p and Top3p (Gangloff *et al.* 1994; Watt *et al.* 1995; Bennett *et al.* 2000). In addition, *sgs1* mutations suppress the slow growth and genomic instability of *top3* mutants (Gangloff *et al.* 1994) but cause slow growth in a *top1* mutant background (Lu *et al.* 1996). Conditional *top2* mutants and *sgs1*Δ null mutants have elevated levels of mitotic and meiotic chromosome missegregation (Holm *et al.* 1989; Watt *et al.* 1995). Both Top2p and Top3p have been postulated to function in the decatenation of newly replicated chromosomes prior to their segregation (Gangloff *et al.* 1994; Spell and Holm 1994; Watt *et al.* 1995). Together, these data suggest that Sgs1p may be involved with one or more topoisomerases in the separation of chromosomes at the late stages of DNA replication (Watt and Hickson 1996).

Srs2p, like Sgs1p, is a DNA helicase with 3′-5′ polarity (Rong and Klein 1993). A mutation in *SRS2* was initially identified as a suppressor of *rad6* and *rad18* UV sensitivity (Aboussekhra *et al.* 1989). Srs2p is proposed to control the sorting of spontaneous DNA lesions into the postreplication repair pathway (Schiestl *et al.* 1990; Rong *et al.* 1991). In the absence of Srs2p, these lesions are purportedly channeled into a *RAD52*-dependent homologous recombination pathway. In addition, *SRS2* has been implicated in nonhomologous end-joining (Hegde and Klein 2000), single-strand annealing (Sugawara *et al.* 2000), and the intra-S checkpoint (Liberi *et al.* 2000).

Yeast cells lacking both the Sgs1 and Srs2 helicases have been reported to be inviable in one study (Lee *et al.* 1999) and slow growing in another (Gangloff *et al.* 2000). Impressively, mutation of genes involved in the initial stages of homologous recombination, including *RAD51*, *-55*, and *-57*, suppresses these defects (Gangloff *et al.* 2000). Therefore, it has been suggested that Sgs1p and Srs2p are required either for the prevention of inappropriate recombination and/or for the processing of recombination intermediates.

We wished to further characterize the causes of premature aging in *sgs1* mutants and determine whether Srs2p also plays a role in the promotion of wild-type longevity. To accomplish this, we have characterized the life spans of *sgs1* and *srs2* mutants in detail. Here, we report that both the *sgs1* and *srs2* mutants have a short life span that can be defined by two separate components. A majority of mutant cells stops dividing stochastically due to mitotic cell cycle arrest, probably due to unrepaired DNA damage. Cells that escape the arrest senesce prematurely due to normal aging processes, most likely caused by ERC accumulation. Genetic analysis using the *sgs1 srs2* double mutant suggests that the mitotic arrest is due to the consequences of inappropriate homologous recombination and can be rescued by diverting DNA repair substrates into other pathways.

MATERIALS AND METHODS

Strains, plasmids, and media: The yeast strains used in this work are detailed in Table 1. The mutant *rad5-535* allele in W303 has been characterized previously (Fan *et al.* 1996). The *sgs1:HIS3* disruption removes nucleotides 317–3752 of the *SGS1* open reading frame (ORF; Gangloff *et al.* 1994), while the *sgs1*Δ*::hisG-URA3* disruption removes nucleotides 481–4026. *SRS2* was disrupted by digesting plasmid pt2Δ2R (Aboussekhra *et al.* 1992) with PstI and then transforming yeast, thereby replacing nucleotides 528–1935 of the *SRS2* ORF with the *LEU2* gene. Disruption of *sgs1* and *srs2* was verified by PCR. *RAD9* was disrupted by integration of a PCR product containing the kanamycin resistance gene (Wach *et al.* 1994) and selection on SC medium containing 300 μg/ml geneticin (GIBCO, Gaithersburg, MD). Disruption of *FOB1* was accomplished by transformation with a PCR product containing the *URA3* or *TRP1* marker gene flanked by 40 nucleotides of *FOB1* sequence. Integration of *SIR2* at *URA3* or *LEU2* was accomplished by transforming cells with p305SIR2 or p306SIR2 digested with *Xcm*I (Kaeberlein *et al.* 1999). The *rad1*Δ*::HIS3*, *rad50*Δ*::HIS3*, *rad51*Δ*::HIS3*, *rad52*Δ*::HIS3*, *rad57*Δ*::HIS3*, and *rad59*Δ*::HIS3* null alleles are described in Park *et al.* (1999). The *hdf1*Δ*::HIS3*, *dnl4*Δ*::HIS3*, *mre11*Δ*::HIS3*, *msh2*Δ*::HIS3*, and *rad18*Δ*::HIS3* alleles were constructed by PCR disruption, removing the entire ORF in all cases.

pSGS1f2 was constructed by PCR amplification of full-length *SGS1* from the yeast genome, including 300 nucleotides (nt) upstream and 350 nt downstream of the coding region. The resulting 5.0-kb fragment was ligated into the polylinker of the ARS-CEN plasmid pRS316. The plasmid complemented an *sgs1* deletion when tested for sensitivity to 0.015% methyl methanesulfonate in agar plates.

All yeast strains were cultured at 30° and grown on YEP medium containing 2% glucose. Diploids were sporulated in 1% potassium acetate medium for 2 days at 30°, and tetrad analysis was performed using standard methods.

Life span and terminal phenotype analysis: Micromanipulation and life span analysis were performed as described previously (Kaeberlein *et al.* 1999). Each experiment consisted of 45–50 mother cells and was carried out at least twice independently. Statistical significance was determined by a Wilcoxon rank sum test. Average life span is stated to be different for $P < 0.05$.

For terminal phenotype analysis, mother cells were observed at least 24 hr after their last division and scored as either unbudded, small budded (diameter of daughter cell less than one-fourth diameter of mother cell), or large budded (diameter of daughter cells equal to or greater than one-fourth diameter of mother cell). Although each life span and terminal morphology distribution figure represents data from a single experiment, each experiment was repeated at least two times with similar results.

Immunofluorescence: Cells were grown to midlog phase and fixed for 20 min in 3.7% formaldehyde. Fixed cells were incubated for 10 min in 0.1 m EDTA with 10 mm dithiotreitol and then washed twice with cold YPD containing 1 m sorbitol. Spheroplasts of fixed cells were obtained by treatment with 0.3 mg/ml zymolyase-100T (ICN Biomedicals) for 30 min at 30°. The cells were then analyzed by immunofluorescence as previously described (Mills *et al.* 1999). DNA was visualized by staining with 4′,6-diamidino-2-phenylindole (DAPI), and microtubules were visualized using the anti-TUB2 antibody BIB2 (a gift from F. Solomon) diluted 1:200 in PBS/1% BSA/0.1% Triton X-100 and a Cy3-conjugated anti-mouse secondary antibody (Amersham, Piscataway, NJ). Digital images were obtained using a CCD camera controlled by OpenLab image acquisition software.

TABLE 1

Yeast strains used in this study

Strain	Genotype
W303	*MAT***a** *ura3-1 leu2-3,112 trp1-1 his3-11,15 ade2-1 can1-100 rad5-535*
W303R	*W303 MAT***a** *RDN1::ADE2 RAD5*
MMY216	*W303 sgs1::HIS3*
MMY217	*W303 srs2::LEU2*
MMY218	*W303 rad9::KAN*
MMY219	*W303 sgs1::HIS3 rad9::KAN*
MMY220	*W303 srs2::LEU2 rad9::KAN*
MMY221	*W303 sgs1::HIS3 srs2::LEU2*
MMY222	*W303 sgs1::HIS3 srs2::LEU2 rad9::KAN*
MMY143	*W303R sgs1::HIS3*
MMY144	*W303R sgs1::HIS3 LEU2/SIR2*
MMY146	*W303R sgs1::HIS3 fob1*Δ*::TRP1*
MMY147	*W303R sgs1::HIS3 fob1*Δ*::TRP1 LEU2/SIR2*
MMY163	*W303R srs2::LEU2 fob1*Δ*::URA3*
PSY316a	*MAT***a** *ura3-52 leu2-3,112 his3-*Δ*200 ade2-101 lys2-801*
PSY316α	*MAT*α *ura3-52 leu2-3,112 his3-*Δ*200 ade2-101 lys2-801*
PPY35	*PSY316a ADE2 hmr*Δ*::ADH1-GFP*
PPY70	*PPY35 sgs1*Δ*::hisG-URA3*
PPY92	*PPY35 rad1*Δ*::HIS3*
PPY96	*PPY35 rad51*Δ*::HIS3*
PPY97	*PPY35 rad57*Δ*::HIS3*
PPY111	*PPY35 rad52*Δ*::HIS3*
PPY143	*PSY316*α *RDN1::ADE2 hmr*Δ*::ADH1-GFP*
PPY217	*PPY143 rad50*Δ*::HIS3*
PPY219	*PPY143 rad59*Δ*HIS3*
MMY249	*PPY143 srs2*Δ*::LEU2*
MMY257	*PPY70* × *MMY249*
MMY258	*PPY35 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2*
MMY259	*PPY143 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2*
MMY282	*PPY35 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2 rad51*Δ*::HIS3*
MMY283	*PPY143 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2 rad51*Δ*::HIS3*
MMY291	*PPY35 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2 rad52*Δ*::HIS3*
MMY292	*PPY143 sgs1*Δ*::hisG-URA3 srs2*Δ*::LEU2 rad52*Δ*::HIS3*
MMY313	*PPY35 hdf1*Δ*::HIS3*
MMY314	*PPY35 dnl4*Δ*::HIS3*
MMY317	*PPY35 mre11*Δ*::HIS3*
MMY319	*PPY35 msh2*Δ*::HIS3*

Strain *W303R* is described in Mills *et al.* (1999). The notation *LEU2/SIR2* indicates that a single copy of the *SIR2* gene has been integrated at the *LEU2* locus.

Cell cycle analysis: Strains were grown to early-log phase (OD_{600} = 0.3) and arrested with α-factor at a final concentration of 13 μg/ml for 3 hr. The α-factor was removed by two washes in YPD and 1-ml aliquots were placed immediately on ice at indicated time points. Cells were processed for fluorescence-activated cell sorting (FACS) as described in Mills *et al.* (1999), except all centrifugations were performed at 3000 × *g* to prevent cell clumping.

RESULTS

Short life span caused by *sgs*1 or *srs*2 mutations: Previously, it was demonstrated that *sgs1* mutants have a mean life span that is ~40% of wild-type strains (Sinclair *et al.* 1997). Therefore, if Srs2p is performing a function similar to Sgs1p, a similar reduction in life span would be expected for *srs2* mutants. To test this, we performed life span analysis on isogenic wild-type, *sgs1*, and *srs2* mutants. Mutation of *SRS2* indeed causes a shortening of life span relative to wild type. The mean life span of an *srs2* mutant is similar to that of an *sgs1* mutant, 10.2 *vs.* 8.6 generations, respectively (Figure 1A).

Different groups have reported that *sgs1 srs2* mutants are either slow growing (Gangloff *et al.* 2000) or inviable (Lee *et al.* 1999). We introduced *sgs1* and *srs2* null mutations into strains of opposite mating type, mated them, sporulated them, and analyzed the meiotic products. Approximately 55% of the time, the double mutant spores were inviable, forming microcolonies of between 2 and 100 cells. The other 45% of spores were able to form slow-growing colonies. Microscopic examination of the colonies revealed a high percentage of large-

budded cells, suggesting that the slow growth was related to the unusual cellular morphology. When cells from these colonies were subjected to life span analysis, their average life span was approximately three generations (Figure 1A).

Cells lacking Sgs1p or Srs2p stop dividing with a terminal morphology distribution distinct from wild-type cells: We wished to characterize the reasons for the premature senescence of *sgs1* and *srs2* cells. Therefore, in addition to counting the number of times that each *sgs1* mother cell divided, we observed the point in the cell cycle at which the mother cell ceased division (hereafter referred to as its terminal morphology). During the course of the life span analysis, we observed that a majority of *sgs1*, *srs2*, and *sgs1 srs2* mother cells stopped dividing with large buds that could not be separated by micromanipulation (Figure 1B). This process appeared to be stochastic, occurring at a rate of ~0.08 per division for the single mutants and 0.25 for the double mutant. In contrast, the majority of wild-type mother cells possessed an unbudded terminal morphology at the end of their life span.

To further investigate whether any cells in the *sgs1* and *srs2* populations senesce in a manner analogous to wild-type cells, we examined the terminal morphologies of both wild-type and mutant cells that had completed various percentages of their normal life spans. The average maximum life span of wild type, as defined by the decile of cells with the greatest number of divisions, is ~32 generations. The average maximum life span of *sgs1* and *srs2* mutants is ~18 generations. Therefore, we examined the terminal morphology distribution, in both mutant strains, of cells that had stopped dividing either early (≤75% of average maximum life span) or late (>75% of average maximum life span; Figure 1C). The wild-type terminal morphology distributions were nearly identical for both populations of cells, with most cells halting division as unbudded or small budded (Figure 1C). However, in *sgs1* and *srs2* mutant cells that stopped dividing early, a majority of cells ceased division with large buds. In striking contrast, late-generation mutant cells were usually unbudded or small budded when they senesced, similar to wild type. This analysis suggests that the distribution of terminal phenotypes observed with *sgs1* and *srs2* cells is composed of two different components. The first component is a G2-arrest that is stochastic and age independent, while the second is a

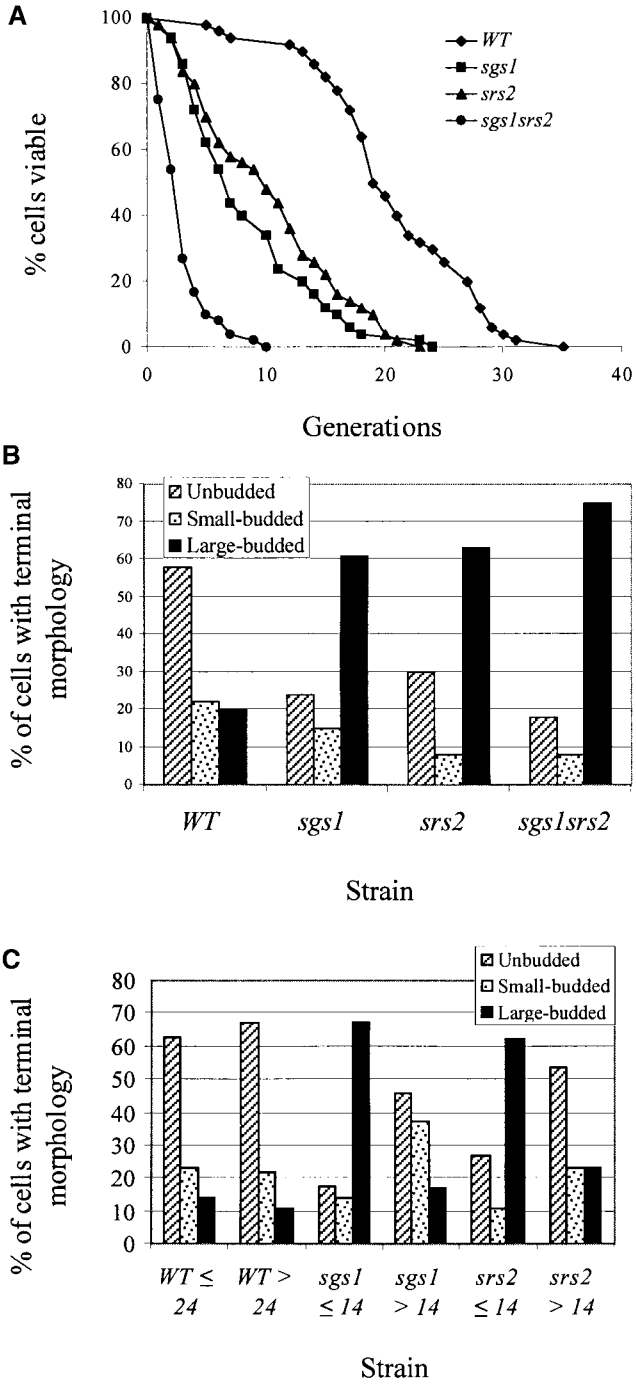


FIGURE 1.—*sgs1*, *srs2*, and *sgs1 srs2* mutants have short life spans and senesce with similar terminal morphologies. (A) Mutation of *SGS1* or *SRS2* results in 60 and 50% reductions in average life span, respectively. The *sgs1 srs2* double mutant displays a synthetic shortening of life span. Mean life spans and the number of mothers examined were as follows: wild type, 20.6 (n = 50); *sgs1*, 8.6 (n = 50); *srs2*, 10.2 (n = 50); and *sgs1 srs2*, 3.0 (n = 47). (B) Wild-type strains senesce mainly as unbudded cells, while *sgs1*, *srs2*, and *sgs1 srs2* mutants senesce with a greater percentage of large-budded cells. Mother cells from the life span in A were observed 24 hr after their last division and scored as either unbudded, small budded, or large budded. (C) *sgs1* and *srs2* mutants have two distinct senescent populations when categorized by terminal morphology distribution. Wild-type, *sgs1*, and *srs2* cells were grouped according to percentage of maximum life span achieved (≤75% and >75%) and their terminal morphologies were plotted. Maximum life span was defined as the average number of divisions completed by the upper decile of cells in each life span. For wild-type cells, 75% of maximum life span corresponded to 24 divisions. For *sgs1* and *srs2* cells, 75% of maximum life span was 14 divisions.

G1-arrest that is age dependent and may be related to wild-type yeast aging.

A portion of *sgs1* and *srs2* mutant cells senesce due to normal causes of aging: Because the terminal morphology distribution of late-generation *sgs1* and *srs2* cells is remarkably similar to the distribution of wild-type cells, we wished to determine whether this subpopulation of helicase mutants was dying due to normal causes of aging. The average and maximum life spans of a wild-type haploid yeast strain can be extended by either deleting the *FOB1* gene or by introducing a second copy of the *SIR2* gene (Defossez *et al.* 1999; Kaeberlein *et al.* 1999). In *fob1* mutants, the life span extension is correlated with a decrease in the accumulation of ERCs, and a similar mechanism of extended longevity has been proposed in the case of *SIR2* overexpression.

Therefore, we tested the effects of the *fob1* mutation and *SIR2* overexpression in our *sgs1* and *srs2* strains. Previously, we reported that deleting *FOB1* gave rise to only a modest increase in the average life span of the *sgs1* mutant (Defossez *et al.* 1999). However, those experiments were confounded by the high rate of stochastic arrest we characterize here. In fact, a reexamination of the earlier data does suggest a possible selective extension in the life span of late generation *sgs1* cells. Life span analysis performed upon several independent isolates of *sgs1 fob1* mutants shows that the life span of *sgs1* mutants was extended by the *fob1* mutation (Figure 2A). However, the extension was primarily observed in the latter half of the life span curve, suggesting that deletion of *FOB1* cannot extend the life span of cells that stop dividing due to the stochastic mechanism.

The overexpression of the *SIR2* gene alone or in combination with the *fob1* deletion extended the maximum life span of the *sgs1* mutant in an identical manner. These life span extensions were reproducible and statistically significant (Figure 2B). Analogously, the latter half of the *srs2* survival curve was extended by deleting *FOB1* or by overexpressing *SIR2* (data not shown). These experiments offer strong evidence that the fraction of *sgs1* and *srs2* cells that avoid the stochastic arrest do age in a manner analogous to wild-type cells and that their life span can be extended by mutations that also extend wild-type life span.

Cell cycle arrest of *sgs1* and *srs2* mutants occurs in mitosis: To further characterize the large-budded phenotype, wild-type, *sgs1*, *srs2*, and *sgs1 srs2* strains were grown to midlog phase, fixed in formaldehyde, and stained with DAPI to visualize DNA. Approximately 15% of the *sgs1 srs2* cells were large budded, with a single nucleus at the neck of the mother cell or in a "bow-tie" conformation stuck between the mother and daughter cells (Figure 3). In the latter case, the nucleus had clearly not yet separated into two distinct nuclei, suggesting that the cells had arrested at a point in mitosis prior to anaphase. This phenomenon was also observed

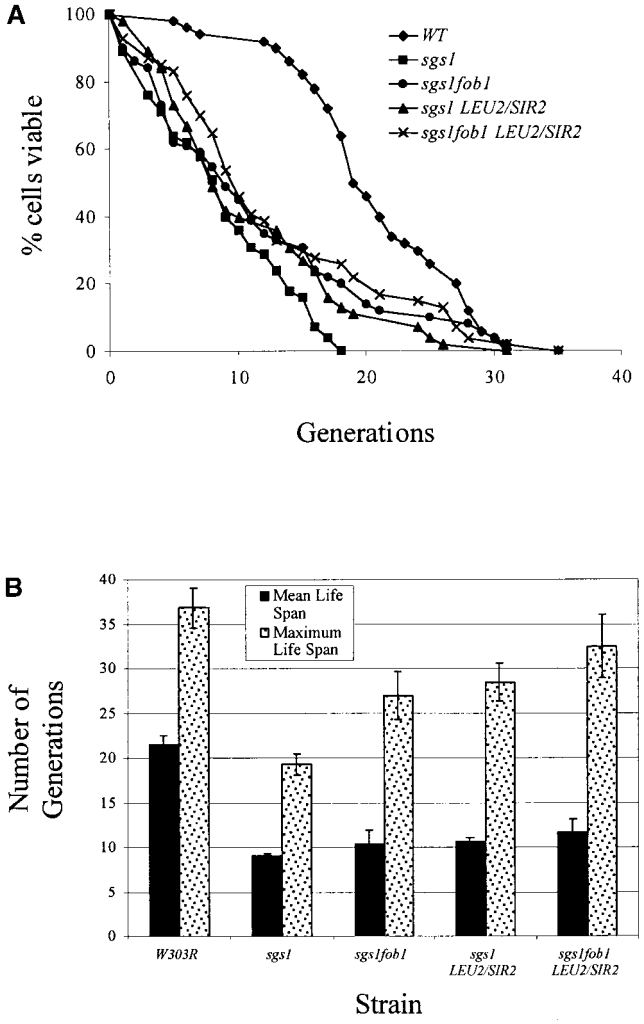


Figure 2.—The maximum life span of an *sgs1* mutant can be extended by deletion of *FOB1* or overexpression of *SIR2*. (A) Deletion of *FOB1* or addition of a second copy of *SIR2* extends the tail of an *sgs1* life span curve. Mean life spans and the number of mothers examined were as follows: wild type, 20.5 (n = 50); *sgs1*, 8.6 (n = 45); *sgs1 fob1*, 11.4 (n = 49); *sgs1 SIR2/LEU2*, 11.0 (n = 45); *sgs1 fob1 SIR2/LEU2*, 12.8 (n = 46). (B) Deletion of *FOB1* or overexpression of *SIR2* has little impact on the average life span of *sgs1* mutants but causes a significant extension of the maximum life span. Each data set represents an average of at least three separate experiments; standard deviation is indicated by a line.

to a smaller extent in *sgs1* and *srs2* single mutants but was noticeably absent from the wild-type population.

To determine the stage of mitosis at which the cells arrested, we costained wild-type and mutant cells with DAPI and antitubulin antibodies to visualize the mitotic spindle. For those mutant cells that had bow-tie nuclei, the spindle was short and extended through the bud neck, characteristic of cells in metaphase (Figure 3). Therefore, it is likely that the early senescence observed in *sgs1* and *srs2* mutants is due to a mitotic checkpoint that is triggered either prior to or at the beginning of chromosome segregation.

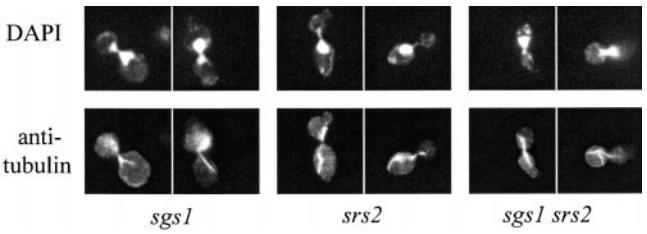


FIGURE 3.—A percentage of *sgs1*, *srs2*, and *sgs1 srs2* cells arrest in mitosis. Indirect immunofluorescence was performed on midlog-phase *sgs1* and *sgs1 srs2* strains. Cells were costained with DAPI to visualize DNA and anti-TUB2 antibodies to visualize microtubules. At least 250 cells from each strain were scored. Percentage of cells that displayed a bow-tie nuclear phenotype with a short mitotic spindle were as follows: wild type, 0%; *sgs1*, 4%; *srs2*, 7%; *sgs1 srs2*, 15%.

The mitotic arrest of *sgs1* and *srs2* mutants is *RAD9* dependent: The cause of the cell cycle arrest in *sgs1* and *srs2* mutants could conceivably be due to DNA damage and/or failure to complete DNA synthesis. In budding yeast, the G2/M DNA damage checkpoint requires the *RAD9* gene (WEINERT and HARTWELL 1988). Therefore, if the cell cycle arrest observed in *sgs1* and *srs2* mutants is due to the presence of DNA damage, deletion of *RAD9* should alleviate the arrest phenotype. Indeed, when a *rad9* null mutation was introduced into the *sgs1*, *srs2*, or *sgs1 srs2* strains and life span analysis was carried out, we observed that the *rad9* mutation partially suppressed the aberrant terminal morphology distribution of *sgs1*, *srs2*, and *sgs1 srs2* mutants (Figure 4A).

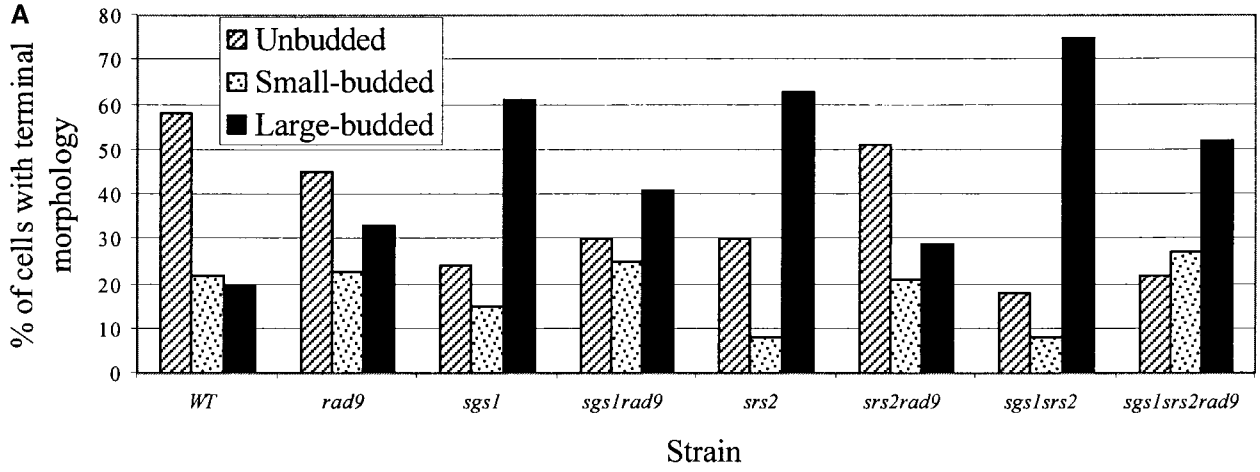
However, the *rad9* mutation did not suppress the life span defects of these strains (Figure 4, B and C). In fact, deletion of *RAD9* further shortened the life span of *sgs1* and *srs2* single mutants. This may be a result of DNA damage that is not repaired prior to completion of mitosis in the *sgs1 rad9* and *srs2 rad9* cells, leading to cell death. Interestingly, the *rad9* mutation slightly increased the life span of an *sgs1 srs2* mutant (Figure 4C). Perhaps in *sgs1 srs2* mutants, irreversible mitotic checkpoint arrest due to DNA damage occurs very frequently. Mutation of *RAD9* bypasses this checkpoint, allowing the cells to divide one or more times, thereby extending their life span.

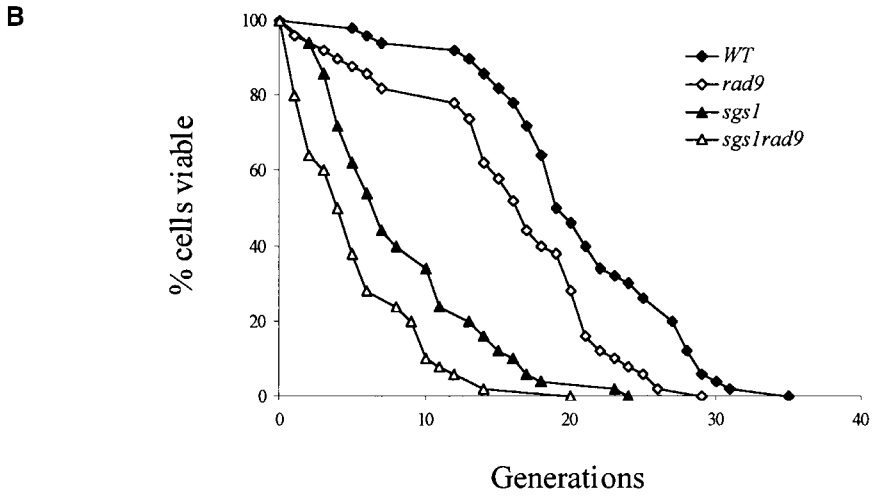
To further confirm that the primary cause of mitotic arrest in *sgs1 srs2* cells is due to DNA damage and not to a gross defect in DNA replication, we monitored cell cycle progression by FACS in populations of cells that were synchronized in G1 by exposure to α-factor. Wild-type and *sgs1* and *srs2* single mutants progressed through S phase by ~40 min after release from α-factor arrest (Figure 5). After ~75 min, many cells had progressed through mitosis, as demonstrated by an increase in the 1n peak. The double *sgs1 srs2* mutant was also able to replicate the bulk of its DNA, although we cannot exclude the possibilities that replication was aberrant or incomplete in a manner that we cannot detect by FACS.

Closer inspection of the FACS plots suggests that many *sgs1 srs2* cells are unable to progress through mitosis. An increase in cells with 1n DNA content is observed in the wild-type population at ~75 and 150 min after release, indicating that they have undergone nuclear division. This increase is qualitatively less noticeable in the *sgs1 srs2* mutant cells, in agreement with the observation that ~25% of these cells arrest during mitosis at each cell division. In addition, a portion of both the single and double *sgs1* and *srs2* mutants have DNA content >2n at time points past 90 min. This could be due to cells that have adapted to the mitotic arrest and are attempting to replicate their DNA in spite of having not completed cell division. Interestingly, *rad9 sgs1 srs2* cells showed a persistent 1n DNA peak. The most probable explanation for this is the presence of dead cells in the culture that underwent an aberrant mitosis prior to α-factor arrest.

Mutation of genes involved in homologous recombination suppresses *sgs1 srs2* slow growth: The bow-tie appearance of the nuclei in terminally arrested *sgs1 srs2* cells suggests that the chromosomes are physically unable to separate. Conceivably, this could occur if the double mutant cells attempt to repair DNA damage using homologous recombination, but are unable to complete the process. By this logic, the cell cycle defect in *sgs1 srs2* cells should be rescued by mutation of genes involved in homologous recombination, including *RAD51*, *RAD52*, *RAD55*, and *RAD57*. To test this idea, we constructed diploid strains heterozygous for *sgs1*, *srs2*, and either *rad51*, *-52*, or *-57* mutations. Following sporulation and tetrad dissection, we determined the genotypes of the resulting haploid spores. Strikingly, mutation of *RAD51*, *-52*, or *-57* fully suppressed the slow growth of an *sgs1 srs2* mutant to the level of the corresponding *rad* single mutant (Figure 6A). In addition, nearly 100% of the triple mutants were viable, as compared to ~45% of the *sgs1 srs2* double mutants (Table 2). These results are in good agreement with recently published data (GANGLOFF *et al.* 2000). Finally, FACS analysis using *sgs1 srs2 rad51* and *sgs1 srs2 rad52* strains demonstrated that these triple mutants were fully able to progress through mitosis without arresting (data not shown). Thus, we postulate that the process of homologous recombination is responsible for the formation of aberrant DNA structures that cause *sgs1 srs2* mutant cells to arrest during mitosis.

Identification of genes with synthetic effects in *sgs1 srs2* mutants: The increased viability and growth rate of *sgs1 srs2 rad51* strains implies that there are pathways, in addition to homologous recombination, that *sgs1 srs2* cells can use to survive. We hypothesized that these additional pathways might involve alternative methods to repair double-strand breaks (DSBs), such as nonhomologous end-joining (NHEJ) or single-strand annealing (SSA). To identify such pathways, we crossed various mutations in genes important for other DNA repair





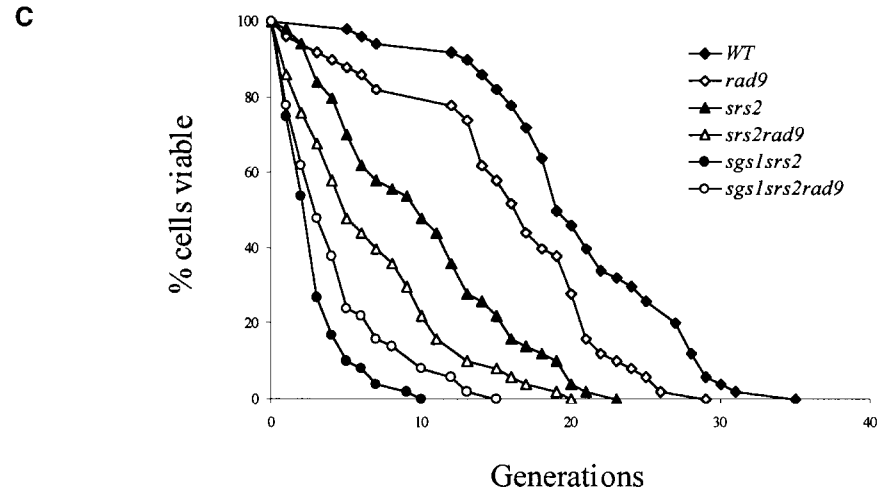


FIGURE 4.—Deletion of *RAD9* suppresses the arrest phenotype of *sgs1*, *srs2*, and *sgs1 srs2* cells but does not promote their longevity. (A) Deletion of *RAD9* partially rescues the mitotic arrest phenotype of *sgs1* and *sgs1 srs2* mutants and fully suppresses the phenotype in *srs2* mutants. *rad9* mutations were introduced into the wild-type, *sgs1*, *srs2*, and *sgs1 srs2* backgrounds and the terminal phenotype distribution of each strain was determined from the life span experiments in B and C. (B) Deletion of *RAD9* further reduces the life span of *sgs1* mutants. Mean life spans and the number of mothers examined were as follows: wild type, 20.6 (n = 50); *rad9*, 15.9 (n = 50); *sgs1*, 8.6 (n = 50); *sgs1 rad9*, 5.3 (n = 50). (C) Deletion of *RAD9* shortens the life span of *srs2* mutants but slightly increases the longevity of *sgs1 srs2* double mutants. Mean life spans and the number of mothers examined were as follows: wild type, 20.6 (n = 50); *rad9*, 15.9 (n = 50); *srs2*, 10.2 (n = 50); *srs2 rad9*, 6.8 (n = 50); *sgs1 srs2*, 3.0 (n = 48); *sgs1 srs2 rad9*, 4.4 (n = 50).

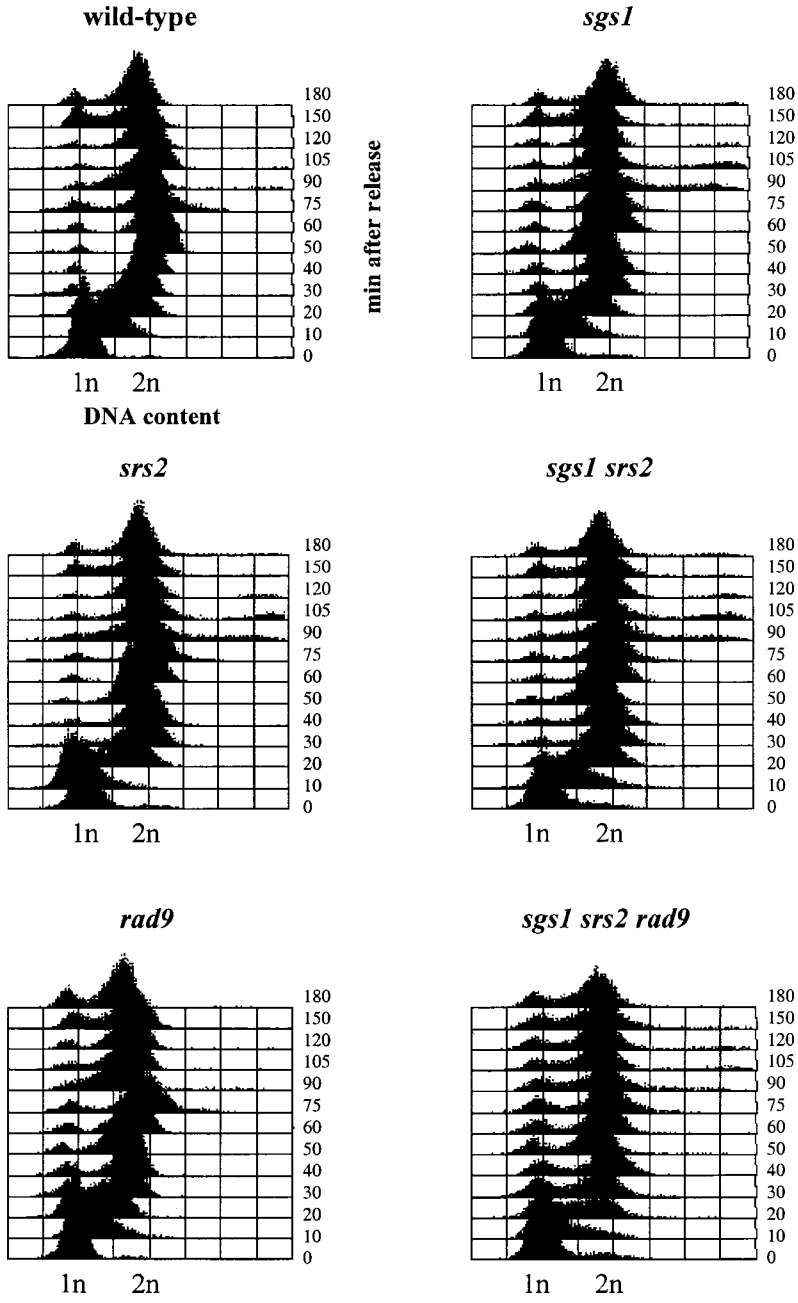


FIGURE 5.—*sgs1 srs2* mutants are proficient in bulk DNA replication but have an apparent mitotic defect that is partially suppressed by the *rad9* mutation. Cells from each strain were arrested in G1 with α-factor, released, and aliquots were removed at indicated time points for FACS analysis.

pathways into *sgs1 srs2* and *sgs1 srs2 rad51* (or *rad52*) mutant backgrounds. The triple and quadruple heterozygous mutants were then sporulated and the meiotic products were analyzed. Because *sgs1 srs2* mutant spores obtained in this manner are viable ~45% of the time, the effects of additional mutations could be readily deduced by scoring their effects on the percentage of viable spores.

First, we tested the hypothesis that *sgs1 srs2* cells could be using a NHEJ pathway to repair DSBs and promote viability. Genes specific to this pathway include *HDF1* and *DNL4* (MILNE *et al.* 1996; WILSON *et al.* 1997). Mutating either of these genes in an *sgs1 srs2* background had no effect on the percentage of viable spores obtained (Table 2). In addition, mutation of *DNL4* in an *sgs1 srs2 rad52* background did not affect the suppression of the *sgs1 srs2* slow growth by *rad52* (Figure 6B). Therefore, *sgs1 srs2* cells cannot be using NHEJ as a significant alternative to homologous recombination for repair of DSBs.

Next, we investigated the effects of mutating other genes involved in recombination. *RAD50*, *MRE11*, and *XRS2* are known to be important for a variety of repair pathways, including homologous recombination resulting in gene conversion, SSA, and NHEJ (reviewed in HABER 1998). Upon sporulation of diploids triply heterozygous for the *sgs1*, *srs2*, and *rad50* or *mre11* mutations, we were unable to obtain viable triple mutants (Table 2). Spores with this inferred genotype divided to form microcolonies of 1–100 cells that stopped dividing

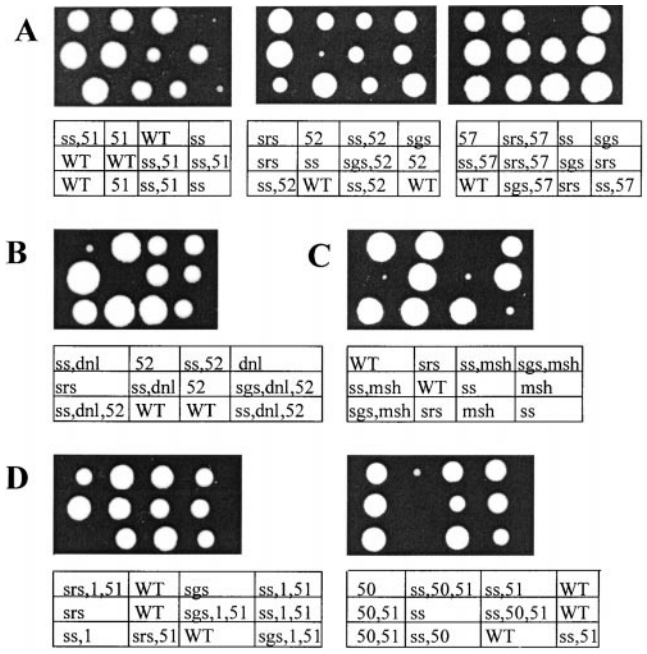


FIGURE 6.—Effects of mutations on *sgs1 srs2* spore viability. Tetrads were dissected for PSY316 derivatives heterozygous for *sgs1*, *srs2*, and other mutations, as indicated. The genotypes of spores were determined by analysis of auxotrophic markers and PCR. Genotypes of spores are abbreviated as follows: 1, *rad1*; 50, *rad50*; 52, *rad52*; sgs, *sgs1*; srs, *srs2*; ss, *sgs1 srs2*; dnl, *dnl4*; msh, *msh2*. Table 2 presents quantification of the data pictured here.

TABLE 2

Genetic modifiers of *sgs1 srs2* spore lethality

Strain	No. of viable spores	No. of inviable spores
sgs1 srs2	165 (44)	213 (56)
srs1 srs2 rad51	68 (99)	1 (1)
sgs1 srs2 rad52	69 (97)	2 (3)
sgs1 srs2 rad57	24 (96)	1 (4)
sgs1 srs2 hdf1	12 (57)	9 (43)
sgs1 srs2 dnl4	11 (44)	14 (56)
sgs1 srs2 dnl4 rad52	9 (100)	0 (0)
sgs1 srs2 mre11	0 (0)	12 (100)
sgs1 srs2 rad50	0 (0)	32 (100)
sgs1 srs2 rad50 rad51	10 (100)	0 (0)
sgs1 srs2 rad50 rad52	15 (100)	0 (0)
sgs1 srs2 rad1	0 (0)	28 (100)
sgs1 srs2 rad1 rad51	16 (94)	1 (6)
sgs1 srs2 rad1 rad52	17 (94)	1 (6)
sgs1 srs2 msh2	12 (39)	19 (61)
sgs1 srs2 rad59	7 (14)	43 (86)
sgs1 srs2 rad51 rad59	18 (95)	1 (5)
sgs1 srs2 rad52 rad59	17 (100)	0 (0)

All strains were constructed in the PSY316 background. Genotypes of viable spores were determined by confirmation of auxotrophic markers and/or PCR. Genotypes of inviable spores were inferred. Numbers in parentheses represent percentages.

with large buds. Given that *srs2 rad50* double mutants grow extremely slowly (HEGDE and KLEIN 2000), it is perhaps not surprising that the triple *sgs1 srs2 rad50* and *sgs1 srs2 mre11* mutants are inviable. As *rad50* and *mre11* are reported to be important for adaptation from G2/M arrest after DSB formation by HO endonuclease (LEE *et al.* 1998), perhaps the *sgs1 srs2 rad50* (or *mre11*) mutants quickly lose viability due to permanent cell cycle arrest.

We also probed the effects of mutating *RAD1*, *RAD59*, and *MSH2*, three genes that are important for the removal of 3′ nonhomologous tails during both gene conversion and SSA (reviewed in PAQUES and HABER 1999). Strikingly, we found that *sgs1 srs2 rad1* mutant spores were never viable, while mutation of *RAD59* resulted in a statistically significant reduction in the percentage of viable spores ($P < 0.05$; Table 2). Furthermore, *sgs1 srs2 msh2* spores that were viable formed smaller colonies than their *sgs1 srs2* counterparts (Figure 6C).

The synthetic lethality of *sgs1 srs2* with the *rad1* and *rad50* mutations was confirmed by transforming the appropriate heterozygous diploids with a plasmid containing *SGS1* and *URA3* (pSGS1f2). After sporulation, several independent *sgs1 srs2 rad1* (or *rad50*) segregants bearing the plasmid were unable to form colonies on 5-fluoroorotic acid (5-FOA) medium (data not shown). Because 5-FOA is lethal to cells expressing the *URA3* gene product, we conclude that *sgs1 srs2 rad1* and *sgs1 srs2 rad1* mutants die because they are unable to lose the *SGS1* plasmid. Thus, *RAD1* and *RAD50* are required for viability in an *sgs1 srs2* mutant.

RAD1 is required for DSB repair by SSA (IVANOV and HABER 1995), while *RAD59* and *MSH2* are important for the efficiency of SSA (SUGAWARA *et al.* 1997, 2000). In addition, mutation of *RAD50* significantly impairs the kinetics of SSA (IVANOV *et al.* 1996). Thus, our results can be explained by a model in which *sgs1 srs2* mutants use SSA as an alternative to homologous recombination for DSB repair (Figure 7). By this model, *sgs1 srs2* cells that attempt to repair DSBs by homologous recombination occasionally arrest permanently due to an inability to either reverse or complete the process. However, *sgs1 srs2* mutants that manage to repair the lesion by SSA are fully able to process the SSA intermediate and do not arrest. Alternatively, *RAD1*, *RAD50*, and *RAD59* may be required for the processing of homologous recombination intermediates after the action of *RAD51*. In their absence, *sgs1 srs2* cells process DSBs to an intermediate that cannot be resolved, resulting in permanent cell cycle arrest.

Surprisingly, introduction of *rad1* or *rad50* mutations into *sgs1 srs2 rad51* or *sgs1 srs2 rad52* backgrounds had no effect on viability or growth rate (Figure 6D). The quadruple mutants are presumably lacking in both homologous recombination and in SSA, but form large colonies. This suggests that other pathways for DSB repair, in addition to SSA, can be mobilized in the ab-

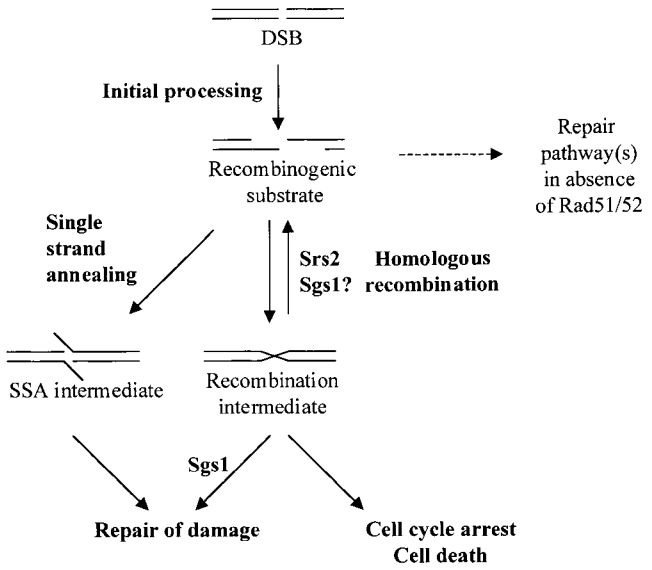


FIGURE 7.—Model for the function of Sgs1p and Srs2p. Double-strand breaks and stalled replication forks require repair. Normally, Srs2p, and perhaps Sgs1p, prevent processing of lesions by the homologous recombination pathway. Sgs1p may also function to resolve homologous recombination intermediates. In an *sgs1 srs2* mutant, repair of DSBs by homologous recombination cannot be completed, resulting in terminal cell cycle arrest and death. Some of the lesions can be repaired by the SSA pathway, resulting in a percentage of *sgs1 srs2* mutants that can form small colonies. In the absence of homologous recombination, additional repair pathways can be used to promote viability.

sence, but not in the presence, of *RAD51/52*-dependent homologous recombination in *sgs1 srs2* mutants (Figure 7).

DISCUSSION

Two components of *sgs1* and *srs2* aging: The data presented here argue that aging in cells lacking the Sgs1 DNA helicase may be more complex than initially proposed (SINCLAIR *et al.* 1997). Careful analysis of the terminal morphology distribution of *sgs1* mutants (Figure 1) reveals that the life span curve of an *sgs1* mutant is a composite of two different phenomena. The early part of the curve can be closely approximated by a stochastic decline in viability, which we believe is due to cell cycle arrest in G2/M. The latter part of the *sgs1* survival curve is composed mostly of cells that stop dividing in G1, suggesting that they senesce due to factors other than the mitotic arrest. Deletion of another DNA helicase, *SRS2*, results in a similar shortening of life span. The extension of life span observed in late generation *sgs1* and *srs2* cells when *FOB1* is deleted or *SIR2* is overexpressed argues that they are aging in a manner analogous to wild-type cells.

Previously, we suggested that *sgs1* mutants age prematurely due to a faster rate of accumulation of ERCs (SINCLAIR and GUARENTE 1997). In fact, we and others have now found that old wild-type and *sgs1* cells that have divided an equal number of times have approximately equivalent levels of circles as quantitated by Southern blotting (HEO *et al.* 1999; our unpublished data). In *sgs1* mutants, if DSBs in the rDNA are largely processed by SSA, the result could well be an increase in recombination, as measured by marker loss, without a concomitant increase in ERCs. In addition, *sgs1* mutants, rather than accumulating more ERCs, may be hypersensitive to ERCs and senesce at levels that are tolerable in wild-type cells. A synthetic effect between the *sgs1* mutation and ERCs is reasonable, since both could place a burden on the DNA replication machinery of the cell. It is still likely that ERCs play a role in aging in *SGS1* wild-type cells, since, first, the ectopic generation of ERCs shortens life span (SINCLAIR *et al.* 1997), and, second, deleting *FOB1* both reduces ERCs and extends life span (DEFOSSEZ *et al.* 1999).

The effect of a *rad9* mutation on *sgs1* and *srs2* life spans: *RAD9* comprises one of two branches of a checkpoint pathway that operates to halt cell cycle progression in the presence of DNA damage (WEINERT and HARTWELL 1988). We observed that deletion of *RAD9* was able to partially suppress the cell cycle arrest observed in *sgs1* and *srs2* cells. However, the viability of *sgs1 rad9* and *srs2 rad9* cells was reduced relative to their *RAD9* counterparts. This is consistent with a model in which lack of Sgs1p or Srs2p creates DNA damage that is recognized by a *RAD9*-dependent checkpoint. The damage could be due to incomplete DNA replication (FREI and GASSER 2000; LIBERI *et al.* 2000) or replication fork pausing (CHAKRAVERTY and HICKSON 1999), which has been shown to cause DSBs in *Escherichia coli* (MICHEL *et al.* 1997).

Upon detection of a DSB, the ensuing cell cycle arrest in *RAD9* cells provides *sgs1* and *srs2* mutants with a period of time in which to repair the DNA damage and resume cell division, presumably with a fully intact genome. Indeed, during life span analysis of both *sgs1* and *srs2* strains, some cells were observed to temporarily arrest and resume cell division up to several hours later. In the absence of Rad9p, *sgs1* and *srs2* mutants do not arrest and divide with unrepaired DNA damage. In many cases, this probably results in catastrophic loss of genetic information and cell death.

Interestingly, the very short life span of the *sgs1 srs2* strain was actually extended by a few generations by deletion of *RAD9*. We suggest that this extension occurs because *sgs1 srs2* cells that would otherwise terminally arrest in G2/M are able to divide one or more additional times in the absence of Rad9p. However, these cells quickly die due to catastrophic DNA damage that is left unrepaired in the absence of the *RAD9* checkpoint.

Possible roles for Sgs1p and Srs2p in homologous recombination: Both DSBs and stalled replication forks, which can be processed into structures that topologi-

cally resemble Holliday junctions (Seigneur *et al.* 1998), can be repaired through a recombinational repair pathway. Sgs1p binds to cruciform structures, including synthetic Holliday junctions, with high affinity and unwinds them (Bennett *et al.* 1999). Therefore, Sgs1p may function, in parallel with Srs2p, to disrupt these Holliday junctions and thereby prevent promiscuous recombination at stalled replication forks or DSBs (Figure 7). In addition, Sgs1p may be required to process the Holliday junctions and resolve the recombination intermediates. Since *srs2* mutants have a greater tendency to repair DSBs by homologous recombination (Schiestl *et al.* 1990), the inability of *sgs1 srs2* cells to disrupt or process these intermediates will be exacerbated.

We propose that the mitotic arrest observed frequently in the *sgs1 srs2* mutant is due to the processing of DSBs by the homologous recombination machinery into chromosomal intermediates that cannot be separated prior to mitosis. These irresolvable structures could be the cause of the bow-tie nuclei observed in G2/M-arrested *sgs1 srs2* cells. In strong support of this model, mutation of genes involved in homologous recombination suppresses the slow growth and mitotic arrest of *sgs1 srs2* cells (Gangloff *et al.* 2000; our data).

The observation that mutation of genes important for SSA, including *RAD50*, *MRE11*, *RAD1*, and *RAD59*, causes synthetic lethality (or reduced spore viability in the case of *RAD59*) in an *sgs1 srs2* background suggests that in the absence of the two helicases, cells may use SSA to repair DSBs. SSA differs from homologous recombination in that Holliday junctions are not formed in SSA (Lin *et al.* 1984). Thus, Sgs1p would not be required for the resolution of SSA intermediates. This model predicts that Sgs1p could play a particularly important role in the rDNA and at telomeres, where the potential for homologous recombination is high. Cells with *sgs1* mutations have an ~7-fold higher rate of marker loss within the rDNA (Gangloff *et al.* 1994). Intriguingly, *sgs1 srs2* mutants have a 40-fold increase in rDNA marker loss (our unpublished data). This increase is partially suppressed by mutation of *RAD51* and is further reduced by mutation of both *RAD1* and *RAD51*. Thus, in cells lacking Sgs1p, perhaps DSBs at the rDNA are processed by SSA instead of by homologous recombination, resulting in deletions and an increased rate of marker loss.

The robust growth of an *sgs1 srs2 rad1 rad52* strain, in which homologous recombination and SSA are absent, demonstrates that additional pathways can be recruited for the repair of DSBs. These pathways are not available to *sgs1 srs2* cells except in the absence of homologous recombination, since *sgs1 srs2 rad1* cells (deficient in SSA) are inviable. Possibly, in an *sgs1 srs2* mutant background, DSBs are initially channeled into the homologous recombination and SSA pathways. If these options are unavailable, cells may employ other repair pathways that operate with slower kinetics.

In summary, the data presented here argue that the short life span of *sgs1* and *srs2* mutants is largely due to an age-independent permanent mitotic arrest that is the result of promiscuous homologous recombination. These findings raise doubts about the classification of *sgs1* as a true premature aging mutant. Nonetheless, further study using the *sgs1* mutant as a model may provide important clues about the underlying causes of human diseases, such as Werner and Bloom syndromes, which are caused by mutations in the RecQ homologues.

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LITERATURE CITED

Aboussekhra, A., R. Chanet, Z. Zgaga, C. Cassier-Chauvat, M. Heude *et al.*, 1989 RADH, a gene of Saccharomyces cerevisiae encoding a putative DNA helicase involved in DNA repair. Characteristics of radH mutants and sequence of the gene. Nucleic Acids Res. **17:** 7211–7219.

Aboussekhra, A., R. Chanet, A. Adjiri and F. Fabre, 1992 Semidominant suppressors of Srs2 helicase mutations of Saccharomyces cerevisiae map in the RAD51 gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. Mol. Cell. Biol. **12:** 3224–3234.

Bennett, R. J., J. A. Sharp and J. C. Wang, 1998 Purification and characterization of the Sgs1 DNA helicase activity of Saccharomyces cerevisiae. J. Biol. Chem. **273:** 9644–9650.

Bennett, R. J., J. L. Keck and J. C. Wang, 1999 Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of S. cerevisiae. J. Mol. Biol. **289:** 235–248.

Bennett, R. J., M. F. Noirot-Gros and J. C. Wang, 2000 Interaction between yeast sgs1 helicase and DNA topoisomerase III. J. Biol. Chem. **275:** 26898–26905.

Chakraverty, R. K., and I. D. Hickson, 1999 Defending genome integrity during DNA replication: a proposed role for RecQ family helicases. Bioessays **21:** 286–294.

Defossez, P. A., R. Prusty, M. Kaeberlein, S. J. Lin, P. Ferrigno *et al.*, 1999 Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell **3:** 447–455.

Fan, H., K. Cheng and H. L. Klein, 1996 Mutations in the RNA polymerase II transcription machinery suppress the hyperrecombination mutant *hpr1*Δ of *Saccharomyces cerevisiae*. Genetics **142:** 749–759.

Frei, C., and S. M. Gasser, 2000 The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci. Genes Dev. **14:** 81–96.

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.

Haber, J. E., 1998 The many interfaces of Mre11. Cell **95:** 583–586.

Hegde, V., and H. Klein, 2000 Requirement for the SRS2 DNA helicase gene in non-homologous end joining in yeast. Nucleic Acids Res. **28:** 2779–2783.

Heo, S. J., K. Tatebayashi, I. Ohsugi, A. Shimamoto, Y. Furuichi *et al.*, 1999 Bloom's syndrome gene suppresses premature ageing caused by Sgs1 deficiency in yeast. Genes Cells **4:** 619–625.

Holm, C., T. Stearns and D. Botstein, 1989 DNA topoisomerase

II must act at mitosis to prevent nondisjunction and chromosome breakage. Mol. Cell. Biol. **9:** 159–168.

Ivanov, E. L., and J. E. Haber, 1995 RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in Saccharomyces cerevisiae. Mol. Cell. Biol. **15:** 2245–2251.

Ivanov, E. L., N. Sugawara, J. Fishman-Lobell and J. E. Haber, 1996 Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics **142:** 693–704.

Jazwinski, S. M., 1999 Molecular mechanisms of yeast longevity. Trends Microbiol. **7:** 247–252.

Kaeberlein, M., M. McVey and L. Guarente, 1999 The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev. **13:** 2570–2580.

Karow, J. K., L. Wu and I. D. Hickson, 2000 RecQ family helicases: roles in cancer and aging. Curr. Opin. Genet. Dev. **10:** 32–38.

Lee, S. E., J. K. Moore, A. Holmes, K. Umezu, R. D. Kolodner *et al.*, 1998 Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell **94:** 399–409.

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.

Liberi, G., I. Chiolo, A. Pellicioli, M. Lopes, P. Plevani *et al.*, 2000 Srs2 DNA helicase is involved in checkpoint response and its regulation requires a functional Mec1-dependent pathway and Cdk1 activity. EMBO J. **19:** 5027–5038.

Lin, F. L., K. Sperle and N. Sternberg, 1984 Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:** 1020–1034.

Lu, J., J. R. Mullen, S. J. Brill, S. Kleff, A. Romeo *et al.*, 1996 Human homologs of yeast DNA helicase. Nature **383:** 678–679.

Michel, B., S. D. Ehrlich and M. Uzest, 1997 DNA double-strand breaks caused by replication arrest. EMBO J. **16:** 430–438.

Mills, K. D., D. A. Sinclair and L. Guarente, 1999 MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. Cell **97:** 609–620.

Milne, G. T., S. Jin, K. B. Shannon and D. T. Weaver, 1996 Mutations in two Ku homologs define a DNA end-joining repair pathway in Saccharomyces cerevisiae. Mol. Cell. Biol. **16:** 4189–4198.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Park, P. U., P. A. Defossez and L. Guarente, 1999 Effects of mutations in DNA repair genes on formation of ribosomal DNA circles and life span in Saccharomyces cerevisiae. Mol. Cell. Biol. **19:** 3848–3856.

Rong, L., and H. L. Klein, 1993 Purification and characterization of the SRS2 DNA helicase of the yeast Saccharomyces cerevisiae. J. Biol. Chem. **268:** 1252–1259.

Rong, L., F. Palladino, A. Aguilera and H. L. Klein, 1991 The hyper-gene conversion hpr5–1 mutation of *Saccharomyces cerevisiae* is an allele of the SRS2/RADH gene. Genetics **127:** 75–85.

Schiestl, R. H., S. Prakash and L. Prakash, 1990 The SRS2 suppressor of rad6 mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the RAD52 DNA repair pathway. Genetics **124:** 817–831.

Seigneur, M., V. Bidnenko, S. D. Ehrlich and B. Michel, 1998 RuvAB acts at arrested replication forks. Cell **95:** 419–430.

Sinclair, D. A., and L. Guarente, 1997 Extrachromosomal rDNA circles—a cause of aging in yeast. Cell **91:** 1033–1042.

Sinclair, D. A., K. Mills and L. Guarente, 1997 Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. Science **277:** 1313–1316.

Spell, R. M., and C. Holm, 1994 Nature and distribution of chromosomal intertwinings in Saccharomyces cerevisiae. Mol. Cell. Biol. **14:** 1465–1476.

Sugawara, N., F. Paques, M. Colaiacovo and J. E. Haber, 1997 Role of Saccharomyces cerevisiae Msh2 and Msh3 repair proteins in double-strand break-induced recombination. Proc. Natl. Acad. Sci. USA **94:** 9214–9219.

Sugawara, N., G. Ira and J. E. Haber, 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.

Sun, H., R. J. Bennett and N. Maizels, 1999 The Saccharomyces cerevisiae Sgs1 helicase efficiently unwinds G-G paired DNAs. Nucleic Acids Res. **27:** 1978–1984.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast **10:** 1793–1808.

Wang, J. C., 1996 DNA topoisomerases. Annu. Rev. Biochem. **65:** 635–692.

Watt, P. M., and I. D. Hickson, 1996 Failure to unwind causes cancer. Genome stability. Curr. Biol. **6:** 265–267.

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.

Watt, P. M., I. D. Hickson, R. H. Borts and E. J. Louis, 1996 SGS1, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics **144:** 935–945.

Weinert, T. A., and L. H. Hartwell, 1988 The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. Science **241:** 317–322.

Wilson, T. E., U. Grawunder and M. R. Lieber, 1997 Yeast DNA ligase IV mediates non-homologous DNA end joining. Nature **388:** 495–498.

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