Mutations in Homologous Recombination Genes Rescue top3 Slow Growth in *Saccharomyces cerevisiae*

Erika Shor, Serge Gangloff,1 Marisa Wagner, Justin Weinstein, Gavrielle Price and Rodney Rothstein2

Department of Genetics and Development, Columbia University College of Physicians & Surgeons, New York, New York 10032-2704

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ABSTRACT

In budding yeast, loss of topoisomerase III, encoded by the *TOP3* gene, leads to a genomic instability phenotype that includes slow growth, hyper-sensitivity to genotoxic agents, mitotic hyper-recombination, increased chromosome missegregation, and meiotic failure. Slow growth and other defects of top3 mutants are suppressed by mutation of *SGS1*, which encodes the only RecQ helicase in *S. cerevisiae*. *sgs1* is epistatic to top3, suggesting that the two proteins act in the same pathway. To identify other factors that function in the Sgs1-Top3 pathway, we undertook a genetic screen for non-*sgs1* suppressors of top3 defects. We found that slow growth and DNA damage sensitivity of top3 mutants are suppressed by mutations in *RAD51, RAD54, RAD55*, and *RAD57*. In contrast, top3 mutants show extreme synergistic growth defects with mutations in *RAD50, MRE11, XRS2, RDH54*, and *RAD1*. We also analyzed recombination at the *SUP4-o* region, showing that in a rad51, rad54, rad55, or rad57 background top3Δ does not increase recombination to the same degree as in a wild-type strain. These results suggest that the presence of the Rad51 homologous recombination complex in a top3 background facilitates creation of detrimental intermediates by Sgs1. We present a model wherein Rad51 helps recruit Sgs1-Top3 to sites of replicative damage.

For every living cell, it is critical to preserve the integrity of genetic material during DNA replication, chromosome segregation, and after DNA damage. Proteins that function in DNA metabolism, such as helicases and topoisomerases, play vital roles in ensuring genome stability. In certain cases, the combination of a helicase and a topoisomerase provides a unique biological function and the interaction between the two is evolutionarily conserved (Duguet 1997; Wu et al. 1999). For example, the archaeabacterial reverse gyrase contains both a helicase and a topoisomerase domain as part of the same polypeptide (Declais et al. 2000; Duguet et al. 2001). Whereas no eukaryotic genome encodes a reverse gyrase, in organisms ranging from yeast to humans, a RecQ family helicase interacts with topoisomerase III both physically and genetically (Gangloff et al. 1994; Goodwin et al. 1999; Bennett et al. 2000; Wu et al. 2000; Fricke et al. 2001). The two proteins and their interaction are critical for maintenance of genome stability (Mullen et al. 2000; Wu et al. 2000). The RecQ family proteins are so classified because they contain a highly conserved helicase/ATPase domain homologous to that of the *Escherichia coli* RecQ helicase (Aaravind et al. 1999). Topoisomerase III is a type IA topoisomerase that can relax negatively supercoiled DNA with a strong preference for single-stranded regions and can decatenate interlinked single-stranded DNA (Kim and Wang 1992; Champoux 2001).

In humans, there are five known RecQ helicase homologs: BLM, WRN, RECQL, RECQL4, and RECQL5. Mutations in *WRN* and *RECQL4* cause Werner (WS) and a subset of Rothmund-Thomson (RTS) syndromes, respectively (Gray et al. 1997; Lindor et al. 2000). Both diseases are associated with a predisposition to certain cancer types, as well as with premature aging (Lindor et al. 2000; Nehlin et al. 2000; Mohaghegh and Hickson 2001). WS cells exhibit high genome instability in the form of increased deletions, translocations, and illegitimate recombination (Shen and Loeb 2000). Mutations in BLM cause Bloom syndrome (BS), a pleiotropic disorder characterized by, among other symptoms, a predisposition to a wide range of cancers (Ellis et al. 1995). At the cellular level, a hallmark feature of BS is an increased rate of sister chromatid exchange (SCE; German 1993). The BLM protein physically interacts with an isoform of topoisomerase III, Top3α, and this interaction is critical for its normal function (Wu et al. 2000). Cells expressing truncated versions of BLM, which are unable to interact with Top3α, have increased SCE rates, reminiscent of those in BS patients (Wu et al. 2000). Although human topoisomerase III has not been implicated in any genetic disorder, deletion of mouse TOP3α results in embryonic lethality (Li and Wang 1998). A

1Present address: CEA de Fontenay-aux-Roses, 92265 Fontenay-aux-Roses, France.

2Corresponding author: Department of Genetics and Development, Columbia University College of Physicians & Surgeons, 701 W. 168th St., New York, NY 10032-2704. E-mail: rothstein@cancercenter.columbia.edu

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mouse with the Top3β isoform deleted is viable but has a shortened life span reminiscent of that in WS and RTS syndrome patients (Kwan and Wang 2001).

In the budding yeast Saccharomyces cerevisiae, loss of topoisomerase III, encoded by the Top3 gene, leads to a pleiotropic phenotype of marked genome instability. Characteristic features of top3 mutants include slow growth, hyper-sensitivity to chemicals that cause DNA lesions or replication arrest, hyper-recombination at the Sup40 and rDNA loci, increased chromosome missegregation during mitosis, and failure to complete meiosis (Wallis et al. 1989; Gangloff et al. 1994, 1996, 1999). Slow growth of top3 mutants is suppressed by mutation of Sgs1, the gene encoding the only RecQ-like helicase in S. cerevisiae (Gangloff et al. 1994). In a wild-type background, sgs1 mutants exhibit a spectrum of defects similar to those in top3 mutants, albeit less severe, as well as a shortened life span (Gangloff et al. 1994; Watt et al. 1995, 1996; Sinclair et al. 1997; Mullen et al. 2000). For most of these defects, sgs1 is epistatic to top3, suggesting that the two proteins act in the same pathway (Gangloff et al. 1994). This conclusion is reinforced by the finding that Sgs1 and Top3 proteins physically interact (Gangloff et al. 1994; Bennett et al. 2000; Fricke et al. 2001). The genetic and physical interactions between Sgs1 and Top3 have led to the following model (Gangloff et al. 1994). In wild-type cells, Sgs1 forms a chromosomal intermediate that Top3 resolves. In top3 mutants, this intermediate persists or is resolved improperly, leading to slow growth and other aspects of the top3 phenotype. In sgs1 mutants this substrate is not created and the need for functional Top3 is alleviated, resulting in suppression of top3 defects.

Analyses of the cell cycle distribution of Sgs1 and Top3 mRNA transcripts and protein products suggest that both Sgs1 and Top3 function during DNA replication. Sgs1 protein levels peak during S phase, decline in G2, and are not detectable during M or G1 (Frei and Gasser 2000). Top3 mRNA levels peak in late G1 and decline in late S/G2 (Cho et al. 1998; Chakraverty et al. 2001). Both Sgs1 and Top3 have been implicated in intra-S-phase checkpoint activation upon DNA damage. For instance, phosphorylation of Rad53, a central S. cerevisiae checkpoint signal transducer, is compromised in top3 mutants and in sgs1 mutants that also carry mutations in Rad24, a gene involved in DNA damage recognition and processing (Frei and Gasser 2000; Chakraverty et al. 2001). Further support for the role of these proteins in DNA replication comes from studies in Xenopus laevis where inactivation or immunodepletion of either of the Sgs1 homologs, FFA-I or xBLM, leads to inhibition of DNA replication in egg extracts (Liao et al. 2000; C. Y. Chen et al. 2001).

Proposed roles of the Sgs1-Top3 complex during S and G2 phases include decatenation of newly replicated sister chromatids, regulation of homologous recombination during DNA replication, and maintenance of stalled replication forks (Gangloff et al. 1994, 2000; van Brabant et al. 2000). In support of its role in chromosome decatenation and segregation, it was shown that Sgs1 physically interacts with topoisomerase II and functions in the topoisomerase II chromosome segregation pathway (Watt et al. 1995). In addition, Harmon et al. (1999) found that E. coli RecQ protein in combination with E. coli or S. cerevisiae topoisomerase III can catenate and decatenate double-stranded DNA circles in vitro.

The idea that the Sgs1-Top3 complex plays a role in homologous recombination is supported by genetic and cell biological studies. Mutation of Sgs1 leads to a severe synthetic growth defect with mutation of another S. cerevisiae helicase, Srs2. Gangloff et al. (2000) found that inactivation of Rad51 fully rescues the sgs1 srs2 synthetic defect. This led to the idea that the defects associated with loss of these two proteins are caused by misregulated or “unrestrained” homologous recombination, suggesting that Sgs1 and Srs2 may regulate homologous recombination during DNA replication (Gangloff et al. 2000). Furthermore, it was shown that Sgs1 physically interacts with Rad51 and that in human cells BLM physically interacts and partially colocalizes with hRad51 during S phase and after DNA damage (Wu et al. 2001). WRN protein was also shown to partially colocalize with RPA and Rad51 in human cells (Sakamoto et al. 2001).

Several observations suggest that the Sgs1-Top3 complex function becomes important when replication forks arrest. Replication forks can stall at programmed pause sites or upon encountering transcription machinery or DNA damage (Rothstein et al. 2000). In S. cerevisiae, both sgs1 and top3 mutants exhibit sensitivity to hydroxyurea (HU), a chemical that stops replication progression by depleting cellular dNTP pools (Frei and Gasser 2000; Mullen et al. 2000). Inactivation of the only RecQ-like helicase in Schizosaccharomyces pombe, Rqh1, also causes HU sensitivity and a defect in recovering from HU-induced S-phase arrest (Murray et al. 1997; Stewart et al. 1997). In addition, deletions of genes encoding the Mus81-Mms4 heterodimer that can efficiently cleave replication fork-like DNA molecules in vitro are inviable in sgs1 and top3 backgrounds, suggesting that Sgs1-Top3 and Mus81-Mms4 complexes have partially overlapping roles in replication fork processing (Kaliraman et al. 2001). In vitro, the Sgs1 protein has 3′ → 5′ helicase activity with a preference for forked substrates (Bennett et al. 1998, 1999). The Sgs1, BLM, and WRN proteins can efficiently migrate synthetic four-way dsDNA junctions that can represent either Holliday junctions or molecules that form after replication fork reversal (Bennett et al. 1999; Constantinou et al. 2000; Karow et al. 2000).

To gain new insights into the function of the Sgs1-Top3 complex and the causes of genome instability and other defects associated with loss of these proteins, we used a genetic approach. Since top3 mutants have a striking slow-
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growing phenotype, we undertook a comprehensive screen for new suppressors of top3 slow growth. Thus far, SGS1 has been the only known gene whose inactivation resulted in alleviation of top3 defects. New top3 suppressor mutations uncovered in the screen fall into several classes. This report will focus on the class consisting of genes involved in homologous recombination and on our investigation of genetic and functional relationships between SGS1, TOP3, and recombination genes. Other mutants identified in the screen will be described elsewhere. Here we show that slow growth of top3 mutants is suppressed by mutations in RAD51, RAD52, RAD54, RAD55, and RAD57. Recently, Oakley et al. (2002) reported similar results. We also show that top3 mutants exhibit an extreme synergistic growth defect in combination with deletion of genes encoding the Mre11-Rad50-Xrs2 (MRX) complex, Rad52, and Rad11.

MATERIALS AND METHODS

Media: Yeast extract-peptone-dextrose (YPD), synthetic complete, and 5-fluoroorotic acid (5-FOA) media were prepared as described (SHERMAN et al. 1986), except twice the amount of leucine was used. Sporulation medium was prepared as described (KLAPHOLZ and ESPOSITO 1982).

Strains: Standard procedures were used for mating, sporulation, and dissection (SHERMAN et al. 1986). Cells were grown at 30°C. Yeast strains used in this study are listed in Table 1. To integrate LEU2 next to SGS1, pWJ1299 was linearized with BglII and transformed into W1588-4C.

Plasmid construction: Plasmid pWJ1189 was made from pWJ212 (WALLIS et al. 1989) via several steps, the last two consisting of subcloning a BamHI/HindIII TOP3 fragment into BamHI/HindIII-cut YCp50 and then subcloning a BglII ADE2 fragment from pRS417 into BamHI-cut YCp50-TOP3 plasmid. Plasmid pWJ1299 was made by amplifying intergenic region YMR188 with primers F-BamHI and R-HinIII-cut YCp50 and then subcloning a BamHI HindIII site of pRS405. (Atomic Energy, Ottawa). Standard procedures were used for mating, sporulation, and dissection (SHERMAN et al. 1986). Cells were grown at 30°C to an OD600 of 1.0. Yeast cells were washed with phosphate buffer, chilled on ice, sonicated, and resuspended in the buffer to a concentration of 3% and cultures were incubated at 30°C. The PCR product with BamHI and HindIII cloning into the BamHI/HindIII site of pRS405.

Isolation of top3A slow growth suppressors: Strain U1619-9D (top3::TRP1 SGS1 pWJ1189 [CEN-TOP3-URA3-ADE2]) was subjected to ethyl methanesulfonate (EMS) mutagenesis as described (LAWRENCE 1991, Chap. 18). Briefly, overnight cultures were washed with phosphate buffer, chilled on ice, sonicated, and resuspended in the buffer to a concentration of 5 × 10⁶ cells/ml. EMS (Aldrich Chemical, Milwaukee) was added to a concentration of 3% and cultures were incubated with shaking at 30°C for 30 min. An equal volume of 10% sodium thiosulfate was added to inactivate EMS. Untreated controls were incubated in parallel with the mutagenesis. Cells were counted and plated in triplicate on -ura and YPD plates to estimate mutagen-induced loss of viability. Viability following EMS treatment after three different rounds of mutagenesis was 50, 65, and 80%. Mutagenized cells were split into 5-25 separate cultures and grown in YPD to facilitate plasmid loss. The following day, 200 ml of each saturated culture was plated on 5-FOA/-trp plates to detect colonies that had lost URA3 expression but had retained the top3::TRP1 disruption. 5-FOA colonies were replica plated to -ade medium to detect colonies that had simultaneously lost URA3 and ADE2 expression, identifying those that had lost pWJ1189. Growth of colonies that are Ura- and Ade- was compared with growth of colonies from an unmutagenized top3A strain. Isolates that grew better than the top3A control were identified as putative top3A slow growth suppressors.

Classifying top3A suppressor mutations: Some top3 suppressor mutations were expected to be due to mutation of SGS1 and have the genotype top3A sgs1 and not top3A SGS1 sup. Since homozygous sgs1/sgs1 diploids are sensitive to 0.03% methyl methanesulfonate (MMS), a complementation test was used to identify top3A slow growth suppressors due to mutation of SGS1. All isolates were crossed to an sgs1A tester strain. The diploids (genotype top3A/+/+ /sgs1A sup/+ or top3A/+/+ /sgs1A/sgs1A) were replica plated to YPD plates containing 0.03% MMS. New mutant alleles of SGS1 fail to complement sgs1A MMS sensitivity and produce diploids unable to grow on 0.03% MMS. Since alleles of SGS1 that suppress top3 slow growth while remaining MMS resistant in a TOP3 background also exist, linkage of the other suppressor mutations to SGS1 was analyzed next (MULLEN et al. 2000). These suppressors were crossed to a strain containing the LEU2 marker integrated next to SGS1. Suppressors unlinked to SGS1-LEU2 were backcrossed at least two more times into a nonmutagenized background to remove potential nonspecific mutations induced by EMS. Suppressor mutations in an otherwise wild-type background were analyzed for their sensitivity to HU, MMS, and ionizing radiation (IR). Those that showed severe sensitivity to IR were crossed to strains containing deletions in RAD51, RAD52, RAD54, RAD55, or RAD57 and complementation of the IR phenotype was assayed. When a suppressor mutation failed to complement IR sensitivity of a radΔ, the corresponding radΔ gene was sequenced in the mutant.

Assaying sensitivity to DNA-damaging agents: HU and MMS were added to the agar medium prior to pouring the plates. Yeast cells were collected from exponentially growing cultures, sonicated, counted, and plated to a quantity of ~300 cells per plate. After 5 days of growth, HU sensitivity of a given strain was evaluated by comparing the size of its colonies to the wild-type control. To determine the MMS sensitivity of a strain, colonies on YPD and MMS plates were counted after 5 days of growth and the number on YPD was taken as 100%. For spot assays, cells were harvested as above and spotted onto plates in a 10-fold serial dilution series, the most concentrated spot containing 10⁵ cells. To assay sensitivity to γ-irradiation, cells were similarly diluted onto YPD plates and exposed to different doses of γ-rays using a Gammacell-220 ³²Co irradiator (Atomic Energy, Ottawa).

SUP4 recombination assay: Determination of deletion frequencies between SUP4 repeats has been described previously (ROTHSTEIN et al. 1987; WALLIS et al. 1989). Briefly, W303 strains contain mutations in the ADE2 and CAN1 genes that are ochre suppressible. The SUP4o dominant allele was linked to a selectable URA3 marker, which was inserted between δ sequences 4 and 5 (see Figure 4A). Upon plating cells onto medium containing 5 μg/ml of adenine and 60 μg/ml of canavanine, it is possible to determine the frequency of cells that have become resistant to canavanine through a recombination event involving the δ sequences. These recombination events lead to the simultaneous loss of the SUP4o and URA3 genes, giving rise to red canavanine-resistant colonies that can no longer grow on medium lacking uracil but can grow on medium containing 5-FOA. The determination of the deletion classes was next performed by probing genomic blots of digested DNA as previously described (ROTHSTEIN et al. 1987). We also developed a PCR-based assay that could distinguish among the seven known SUP4 deletion classes. Four PCR primers, A (GGACAAACATAACAGCGCAG), B (GTGCGAAAA CTTACACGTAGGGGAG), and
D (TCACAACGGCATAGAAGCC), from regions adjacent to δ 1, 2, 3, and 5, respectively, were designed. PCR products were obtained from two different sets of reactions and were compared both before and after digestion with XhoI. For example, primer pairs A/B and C/D amplify fragments of 781 and 1259 bp for class I or II events. Upon digestion with XhoI, the C/D product from class I events generates two fragments (303 and 956 bp) while class II events generate three fragments (242, 303, and 714 bp). In a similar fashion, all seven deletion classes could be unambiguously assigned.

RESULTS

Screen for non-sgs1 suppressors of top3 slow growth: We performed an EMS mutagenesis to screen for new

<table>
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<th>Strain*</th>
<th>Genotype</th>
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<td>W1588-4C</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</td>
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<tr>
<td>W1588-4A</td>
<td>MATx ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</td>
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<tr>
<td>U1619-4D</td>
<td>MATa top3::TRP1 pWJ189 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2Δ</td>
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<tr>
<td>W3209-3C</td>
<td>MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 met15Δ</td>
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<tr>
<td>W3266-10B</td>
<td>MATa svg1::His3 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 met15Δ</td>
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<tr>
<td>W3209-10C</td>
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<td>W3210-18B</td>
<td>MATa rad52::His5 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 met15Δ</td>
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<tr>
<td>W3211-29B</td>
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<td>W3912-6D</td>
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<td>W3269-7B</td>
<td>MATa SG51::LEU2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</td>
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(continued)
SGS1 suppressors were analyzed, 34 of which were due to mutations in SGS1. Materials and methods. To examine whether deletion of these RAD genes has the same effect on top3 slow growth as the point mutations isolated in the screen, the corresponding rad deletion mutants were crossed to a top3 strain. The diploids were sporulated and the tetrads were dissected. Growth rates of colonies that had lost both URA3 and ADE2 expression were compared with that of an unmutagenized top3 strain. Putative top3 slow growth suppressors were identified as growing faster than their unmutagenized counterparts (Figure 1A).

Until this report, SGS1 had been the only known gene whose inactivation resulted in suppression of top3 slow growth (Gangloff et al. 1994). Moreover, all 25 spontaneous top3 slow growth suppressors identified in our laboratory were shown to be tightly linked to SGS1 (our unpublished data). To investigate whether the new suppressor mutations were allelic to SGS1, complementation tests and genetic crosses were performed (see materials and methods for details). Mutations that complemented sgs1 MMS sensitivity and were unlinked to SGS1 were analyzed further (Figure 1B). Overall, 53 top3 suppressors were analyzed, 34 of which were due to mutations in SGS1.

Mutations in homologous recombination genes suppress top3 slow growth and sensitivity to DNA-damaging agents: The phenotype of 19 suppressor mutations unlinked to SGS1 was analyzed in an otherwise wild-type background. Since both Sgs1 and Top3 function in DNA metabolism and genome stability, it was possible that new top3 suppressor mutations would also identify genes involved in these processes. Thus, HU, MMS, and IR sensitivities of the new mutants were tested. We observed that nine strains bearing suppressor mutations were sensitive to all three of these agents. Their sensitivity to IR indicated that they are defective in double-strand break (DSB) repair. Thus, standard complementation tests were used to determine whether the IR-sensitive mutants were allelic to members of the RAD52 epistasis group. By this analysis, we identified 1 rad51, 1 rad52, 3 rad54, 3 rad55, and 1 rad57 mutants. For each mutant, the appropriate RAD gene was sequenced; Table 2 lists the mutations isolated in this screen. In addition to the rad mutants, 10 other non-sgs1 mutants were identified that rescue top3 slow growth. These mutants will be described in a separate article.

To examine whether deletion of these RAD genes has the same effect on top3 slow growth as the point mutations isolated in the screen, the corresponding rad deletion mutants were crossed to a top3 strain. The diploids were sporulated and the tetrads were dissected. Growth rates of top3, top3 rad mutants and appropriate controls were examined in liquid YPD medium and the results are summarized in Figure 2A. A wild-type strain has a doubling time of ~90 min, while an isogenic top3 strain has a doubling time of ~260 min. Deletion of SGS1 in a top3 background reduces the doubling time to 113 min, which is similar to that of an sgs1 TOP3 strain, illustrating that sgs1 is epistatic to top3 for growth. We

<table>
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<td>J599¹</td>
<td>MATa SUP4::URA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535</td>
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<td>J60¹</td>
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<td>MATa SUP4::URA3 sgs1-25 top3-2::HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535</td>
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<td>MATa SUP4::URA3 rad54::LEU2 top3-5::TRP1 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535</td>
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<td>MATa SUP4::URA3 sgs1-25 top3-5::TRP1 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535</td>
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</tr>
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</table>

* All strains are derivatives of W1588 (Zou and Rothstein 1997), a RAD5 derivative of W303 (Thomas and Rothstein 1989), unless noted otherwise.

* This study.

Top3 slow growth suppressors, including possible hypomorphic mutations in essential genes. We had observed previously that spontaneous mutations (sup) that cause faster growth occasionally arise in a top3 background. When such an event occurs early in a top3 culture, most cells in the culture are top3 sup. To avoid these jackpot events prior to mutagenesis, the top3 deletion was propagated in a strain containing a TOP3 plasmid marked with URA3 and ADE2. This serves two purposes: (1) the strain becomes top3 only upon plasmid loss; (2) the presence of the plasmid can be scored both by 5-FOA sensitivity and by color, as adenine prototrophs yield white colonies. The top3 pTOP3-URA3-ADE2 strain was treated with EMS to induce mutations and was subsequently grown under conditions that allow plasmid loss. Growth rates of colonies that had lost both URA3 and ADE2 expression were compared with that of an unmutagenized top3 strain. Putative top3 slow growth suppressors were identified as growing faster than their unmutagenized counterparts (Figure 1A).

This study.
found that deletion of \textit{RAD}51, -54, or -55 in a \textit{top3}\textDelta background reduces doubling time to \textasciitilde155 min. This is greater than the doubling time of the corresponding \textit{rad} mutants in a \textit{TOP3} background, indicating that their suppression of \textit{top3} slow growth is partial. Deletion of \textit{SGS1} in \textit{top3 rad51}, \textit{top3 rad54}, or \textit{top3 rad55} mutants improves the growth rate to that of an \textit{sgs1} mutant, indicating that the slow growth of a \textit{top3 rad} mutant is caused by \textit{SGS1}. A catalytically inactive allele of \textit{RAD54}, \textit{rad54-K341A}, also suppresses \textit{top3}\textDelta slow growth (data not shown). Unlike \textit{rad51}, \textit{rad54}, and \textit{rad55} mutants, the \textit{rad52} strain is slow growing on its own, with a doubling time of 130 min. Deletion of \textit{RAD52} in a \textit{top3}\textDelta background reduces the doubling time from 260 to \textasciitilde190 min. Interestingly, in an \textit{sgs1}\textDelta background, deletion of \textit{RAD52} leads to a synergistic decrease in growth rate and a doubling time of \textasciitilde170 min. This observation suggests that \textit{SGS1} becomes especially important for normal growth in the absence of \textit{Rad52} and vice versa. Removal of \textit{TOP3} does not further reduce the growth rate of an \textit{sgs1 rad52} double mutant. In summary, these results demonstrate that a loss-of-function mutation in a gene affecting homologous recombination can improve the growth rate of a \textit{top3} strain.

In addition to slow growth, \textit{top3} mutants are highly sensitive to the DNA-damaging agents HU and MMS (Chakraverty \textit{et al.} 2001; Figure 2, B and C). We tested whether homologous recombination contributes to this sensitivity by examining the phenotype of \textit{top3}\textDelta mutants carrying deletions in \textit{RAD51}, -52, or -55. To measure HU or MMS sensitivity, the strains were pregrown in liquid YPD medium, sonicated, and plated onto YPD plates containing different concentrations of HU or MMS. Presence of 10 mm HU in the medium severely retards the growth of \textit{top3} mutants, resulting in formation of extremely small colonies after 5 days (Figure 2B). Deletion of \textit{SGS1}, \textit{RAD51}, or \textit{RAD55} in \textit{top3} mutants improves their ability to grow on HU-containing medium. On the other hand, deletion of \textit{RAD52} does not rescue HU sensitivity of \textit{top3} mutants (Figure 3B). Plating of \textit{top3} mutants onto medium containing MMS results in their decreased survival (colony-forming ability) compared to plating onto medium without MMS (Figure 2C). Deletion of \textit{SGS1}, \textit{RAD51}, or \textit{RAD55} in a \textit{top3} background rescues this defect, with \textit{sgs1}\textDelta being the best suppressor and \textit{rad51}\textDelta the weakest (Figure 2C). In contrast, deletion of \textit{RAD52}, which leads to significant MMS sensitivity on its own, does not rescue \textit{top3} MMS sensitivity (Figure 2C and Figure 3D).

Previously, Wu \textit{et al.} (2001) reported that \textit{RAD51} and \textit{SGS1} are in the same epistasis group for HU and MMS sensitivity. These researchers measured the growth inhibition of \textit{sgs1}, \textit{rad51}, and \textit{sgs1 rad51} mutants during transient exposure to 15 mm HU or 0.002% MMS. In addition, the same strains were plated in 10-fold dilutions and grown for 2–3 days on plates containing the same concentrations of the drugs. Here we explore further the genetic relationship between \textit{SGS1} and the homologous recombination pathway by measuring the sensitivities of \textit{sgs1}, \textit{rad51}, \textit{rad52}, \textit{rad54}, \textit{rad55}, and \textit{sgs1 rad} mutants to different concentrations of HU and MMS using the protocol described in \textit{Materials and Methods}. In our genetic background, we found that \textit{sgs1} and the \textit{rad} mutants can grow on higher concentrations of HU and MMS than those mutants used above, although they are clearly sensitive to these agents. For example, single cell plating experiments show that 30 mm HU retards the

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**TABLE 2**

<table>
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<tr>
<td>\textit{rad51}</td>
<td>Ala252Thr</td>
</tr>
<tr>
<td>\textit{rad52}</td>
<td>Asp164Asn</td>
</tr>
<tr>
<td>\textit{rad54}</td>
<td>Gly340Ser, Ser654Leu, Gly745Ser</td>
</tr>
<tr>
<td>\textit{rad55}</td>
<td>Gln78Lys, Met175Ile, Glu201Asn, Gln268Stop</td>
</tr>
<tr>
<td>\textit{rad57}</td>
<td>Gly298Lys</td>
</tr>
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</table>

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**FIGURE 1**—Isolation of non-\textit{sgs1} suppressors of \textit{top3}\textDelta slow growth. (A) After EMS mutagenesis, colonies that lost pTOP3-\textit{URA3-DE2} were streaked on YPD next to an unmutagenized \textit{top3}\textDelta strain. A representative plate is shown with arrowheads marking mutants that were picked as \textit{top3}\textDelta slow growth suppressors. (B) Mutations in \textit{SGS1} were ruled out by complementation analysis. \textit{top3}\textDelta slow growth suppressor candidates (\textit{sup}) were crossed to an \textit{sgs1}\textDelta strain and the diploids were replica plated to 0.03% MMS. Candidates that complement \textit{sgs1}\textDelta, indicated by arrowheads, were analyzed further.
Suppressors of top3 Slow Growth

Figure 2.—Disruption of homologous recombination genes rescues top3 defects. All experiments shown were performed with strains containing deletions of the indicated genes. (A) Deletion of SGS1, RAD51, RAD52, RAD54, or RAD55 improves the growth rate of a top3 mutant. Doubling times of the indicated strains in rich medium are shown. (B) Deletion of SGS1, RAD51, or RAD55 rescues HU sensitivity of a top3 mutant. All plates were scanned 5 days after plating, which allowed the top3 colonies on YPD to attain the same size as the other strains. The very small top3 colonies on 10 mM HU are visible on the enlarged section of the plate, which is shown with enhanced contrast for clarity. (C) Deletion of SGS1, RAD51, or RAD55, but not RAD52, rescues MMS sensitivity of the top3 mutant. Survival curves of the indicated mutants are shown. As described in MATERIALS AND METHODS, log-phase cells growing in rich liquid medium were harvested, sonicated, and plated to the indicated concentrations of MMS (abscissa). After 5 days, survival was measured by counting colonies. The number of colonies on plates containing no MMS was taken as 100%. Error bars represent the standard deviation.

growth of sgs1, rad51, rad54, and rad55 single mutants but not that of an isogenic wild-type strain (Figure 3A; data not shown). Interestingly, sgs1 rad51, sgs1 rad54, and sgs1 rad55 double mutants grow even more slowly on 30 mM HU, forming smaller colonies after 5 days of growth than those of either the sgs1 or the rad mutants alone (Figure 3A; data not shown). The rad52A strain is more sensitive to HU than the rad51Δ, rad54Δ, or rad55Δ strains, and rad52A HU sensitivity is also exacerbated by deletion of SGS1 (Figure 3B). These data indicate that SGS1 and the RAD genes participate in different pathways that repair HU-induced damage.

Whereas addition of HU to the medium retards the growth of sgs1 and rad mutants, addition of MMS to the medium results in decreased survival (colony-forming ability) of these strains compared to an isogenic wild-type strain (Figure 3, C and D). Deletion of SGS1 in a rad51, rad52, rad54, or rad55 background even further reduces survival in the presence of MMS (Figure 3, C and D, and data not shown). This result suggests that, similar to their roles in HU resistance, SGS1 and the RAD genes participate in different pathways that repair MMS-induced DNA damage.

Frequency and mechanisms of recombination at the SUP4 locus in top3Δ mutants: The SUP4-0 gene in S. cerevisiae encodes a tyrosine tRNA ochre suppressor surrounded by five 6 sequences derived from long terminal repeats of the yeast Ty transposon (Rothstein et al. 1987; Figure 4A). These 6 repeats occur in both direct and inverted orientation and are from 71 to 97% homologous to each other. The SUP4 region may be difficult to replicate as it contains natural replication pause sites (e.g., three tRNA genes) as well as the 6 repeats. Such elements may promote formation of recombinogenic lesions (i.e., chromosomal structures that lead to increased genetic recombination) during DNA replication. In wild-type cells, these lesions occur at low frequencies and are normally repaired by rearrangement-free means, such as gene conversion (GC) using the sister chromatid as a template (Rothstein et al. 1987; McDonald and Rothstein...
If the lesions are repaired by GC between misaligned sister chromatids or by single-strand annealing (SSA), this can lead to a deletion of the SUP4-o gene (Rothstein et al. 1987). Insertion of a URA3 marker adjacent to SUP4-o simplifies the detection of such deletions as they result in the simultaneous loss of both genes. In wild-type cells, seven distinct deletion classes arise and they can be distinguished by genomic blotting or PCR (Rothstein et al. 1987; Figure 4B; Materials and Methods). Five classes that are either unassociated (classes I and II) or associated (classes III, IV, and VII) with crossing over (CO) arise via GC. The other two deletion classes (V and VI) are thought to arise primarily by direct repeat recombination via SSA between δ sequences 3 and 5, although other mechanisms for their formation (e.g., unequal sister chromatid exchange of break-induced replication, or BIR) are also possible (Rothstein et al. 1987). For simplicity, we refer to classes V and VI as the SSA classes.

We analyzed the distribution of deletion classes and the overall deletion frequency in top3, sgs1, rad, and relevant double mutants to understand the role of these proteins in GC and SSA and to explore the reasons for top3 rescue by rad mutations. Originally, TOP3 was identified as a mutation that leads to increased SUP4-o deletion formation (Wallis et al. 1989). SUP4-o marker loss is increased 90-fold in top3 mutants compared to wild-type cells (Figure 4B). Mutation of SGS1 also leads to hyper-recombination at SUP4-o, with a 16-fold increase in deletion formation (Figure 4B). In a top3 background, mutation of SGS1 reduces SUP4-o recombination from a 90-fold increase to a 35-fold increase, supporting the notion that Sgs1 functions upstream of Top3. Table 3 lists the distribution of deletion classes in various mutant backgrounds. Both GC and SSA classes are seen in top3 and sgs1 mutants, suggesting that neither protein is essential for either GC or SSA. In top3 mutants, however, among the GC classes, the proportion of CO classes III and IV is increased and the proportion of non-CO classes I and II is decreased relative to wild type (Table 3).

Deletion of RAD51, -54, -55, or -57 leads to a 2- to 7-fold overall increase in SUP4 recombination. However, in these mutants, the GC classes I–IV and VII are almost entirely abolished and virtually all deletions belong to the SSA classes V and VI, due to hyper-recombination between δ sequences 3 and 5 (Figure 4B; Table 3). These results support previous reports that mutations in RAD51 epistasis group genes lead to a severe reduction of GC but cause an increase in recombination between direct repeats (McDonald and Rothstein 1994;...
we tested the effect of deleting genes of the RAD50 group in a top3 mutant background. Appropriate crosses were performed and tetrads dissected. We found that deletion of MRE11, RAD50, or XRS2 (MRX) in a top3Δ background has a striking deleterious effect on viability and growth (Figure 5A, data not shown). In each case, double mutants, when viable, form only microcolonies.

This synthetic defect is most severe for the top3 mre11 mutants, which are usually inviable. Similar to other top3 defects, top3 mrx mutants are partially rescued by deletion of SGS1 or RAD51 (Figure 5A).

We also investigated the genetic interaction between MRX and SGS1. We found that deletion of SGS1 exhibits a synergistic slow growth with all three components of the MRX complex, the most severe synthetic defect being with mre11Δ. Figure 5B shows the doubling times of the appropriate single and double mutants in liquid YPD. Since deletion of RAD51 partially rescues top3 slow growth, we tested whether it would also rescue the sgs1 rad50 synthetic slow growth. We found that rad51Δ indeed reduces the doubling time of an sgs1 rad50 mutant from 202 to 171 min (Figure 5B). Additionally, sgs1, rad50, rad51, and the double- and triple-mutant combinations were tested for HU, MMS, and IR sensitivity (Figure 5C). We found that sgs1 rad50 mutants are more sensitive to HU and MMS than either single mutant but exhibit IR sensitivity that is similar to that of the rad50 mutant. Lastly, deletion of RAD51 does not rescue these synthetic defects.

Exploring the contribution of Rdh54, Rad59, and Rad1 in the absence of Sgs1-Top3: Rdh54 and Rad59 also belong to the RAD52 epistasis group (Paques and Haber 1999). RDH54 encodes a Rad54 homolog thought to be involved in homologous recombination in diploid cells (Klein 1997; Shinohara et al. 2000). RAD59 encodes a Rad52 homolog whose inactivation results in a strong
TABLE 3
Distribution of deletion classes in different strain backgrounds

<table>
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<tr>
<th>Class</th>
<th>Type</th>
<th>Wild type</th>
<th>top3</th>
<th>sgs1</th>
<th>sgs1 top3</th>
<th>rad52</th>
<th>rad52 top3</th>
<th>rad51</th>
<th>rad51 top3</th>
<th>rad54</th>
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<tr>
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<td>7</td>
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Figure 4A shows the structure of the SUP4 region and the different deletion classes. For each strain background, the number corresponds to the percentage of deletions that belong to a particular class.

Gene conversion unassociated with crossing over.
Gene conversion associated with crossing over.
Single-strand annealing.

The requirement of MRX and Rdh54 functions underscores the importance of Rad51-independent processes for survival in the absence of Top3. The MRX complex is involved in at least two Rad51-independent processes: nonhomologous end joining (NHEJ) and SSA (Ivanov et al. 1996; Lewis and Resnick 2000; L. Chen et al. 2001). Therefore, we decided to examine genetically the contribution of each of these processes to normal viability and growth in a top3 background.

NHEJ is a homology-independent error-prone mechanism of DSB repair that ligates two broken DNA ends. This process is dependent on the Ku70 and Ku80 proteins, encoded in yeast by YKU70 and YKU80, DNA ligase 4, and the MRX complex (Lewis and Resnick 2000; L. Chen et al. 2001). To address whether NHEJ plays a role in resolving chromosomal intermediates that arise in top3 mutants, we constructed a top3Δ yku70Δ double mutant. Deletion of YKU70 had no detrimental effect in a top3Δ background, indicating that NHEJ is likely not involved in the repair of chromosomal structures formed in the absence of Top3 (Figure 6D).

A DSB can be processed by SSA if there are regions of homology on both sides of the break (Paques and Haber 1999). SSA requires the Rad11/Rad10 heterodimer, which functions as an endonuclease and removes single-stranded DNA tails adjacent to the annealed regions (Ivanov and Haber 1995). We previously reported that deletion of RAD1 reduces the size of top3Δ spore colonies (Bailis et al. 1992; Figure 6C). We now find that although top3Δ rad1Δ mutants grow extremely poorly, they form slightly larger colonies than those of top3Δ rad50Δ mutants. Also, unlike top3Δ rad50Δ mutants whose synthetic defect is weakly suppressed by deletion of SGS1, the size of top3Δ rad1Δ colonies is almost fully restored to wild type by sgs1Δ (compare Figures 5A and 6C). Deletion of RAD51 also rescues the top3Δ rad1Δ synthetic defect (data not shown). To investigate whether the MRX complex and Rad11 function in the same processes in the absence of Top3 (e.g., SSA), we created a diploid heterozygous for top3Δ, rad50Δ, rad1Δ, and sgs1Δ and dissected 66 tetrads. Assuming 100% spore viability, we expected ~16 top3Δ rad1Δ rad50Δ triple mutants and the same number of quadruple mutants (top3Δ rad50Δ rad1Δ sgs1Δ). Although 11 quadruple mutants formed easily discernible microcolonies, no microcolonies were observed for the triple top3Δ rad1Δ rad50Δ mutant. Thus, while simultaneous
deletion of RAD1 and RAD50 in an otherwise wild-type background does not result in a synthetic growth defect (data not shown), deletion of both RAD1 and RAD50 in a top3Δ background leads to lethality that is rescued by deletion of SGS1. This observation suggests that Rad50 and Rad1 have nonoverlapping functions in the absence of Top3.

Finally, to complete our investigation of the functions of the RAD52 epistasis group genes in the absence of Sgs1, we explored the effect of deleting RAD59 or RDH54 in an sgs1Δ background. Since deletion of RAD1 shows a synergistic defect with top3Δ, we also combined rad1Δ with sgs1Δ to investigate whether Rad1 has important functions in the absence of Sgs1. Measurements of growth rates in liquid medium showed that none of these mutants are slow growing on their own or retard growth further in the absence of Sgs1 (data not shown). We also tested the effects of deleting RAD59, RDH54, or RAD1 on HU and MMS sensitivity in an otherwise wild-type or sgs1Δ background. Figure 6, E and F, summarizes these results. The rad59Δ strain shows mild sensitivity to HU and intermediate sensitivity to MMS at the concentrations tested. In an sgs1Δ background, deletion of RAD59 results in additional sensitivity to both agents, suggesting that Rad59 is involved in Sgs1-independent and HU- and MMS-resistant pathways. The rdh54Δ mutant behaves similarly to the wild-type strain with respect to HU and MMS resistance at the concentrations tested. However, in an sgs1Δ background, deletion of RDH54 results in increased sensitivity to both agents, indicating that Rdh54 function becomes important for HU/MMS resistance in the absence of Sgs1. Similar to rdh54Δ, the rad1Δ strain does not display HU or MMS sensitivity at the concentrations tested. However, the sgs1 rad1Δ mutant is slightly more HU sensitive and significantly more MMS sensitive than the sgs1 mutant, showing that Rad1 becomes important for resistance to these agents in the absence of Sgs1.

**DISCUSSION**

**Mutation of the Rad51 homologous recombination complex rescues top3 defects:** Our results demonstrate that RAD51, RAD54, RAD55, and RAD57 contribute to slow growth and HU and MMS sensitivity in a top3Δ background. Genetic and biochemical studies indicate that, at the molecular level, Rad51 catalyzes the invasion of ssDNA into a homologous duplex and is aided by the
DNA annealing protein Rad52 and the Rad55/Rad57 heterodimer (Sung et al. 2000). Rad54, a member of the Swi2/Snf2 family of chromatin-remodeling proteins, presumably makes DNA more accessible for the recombination reaction (Sung et al. 2000). Since mutations in these genes rescue top3 defects, certain properties or functions of the homologous recombination complex comprised by these proteins must be detrimental in the absence of Top3.

RAD51, RAD54, RAD55, and RAD57 belong to the RAD52 epistasis group. Deletion of RAD52, a central S. cerevisiae homologous recombination gene, results in a decreased growth rate and resistance to HU and MMS on its own, but rad52Δ also partially rescues top3 slow growth. The RAD52 epistasis group has been genetically subdivided into two branches: the RAD51 pathway (RAD51, RAD54, RAD55, and RAD57) and the RAD50 pathway (MRX, RAD59, and RDH54). Processes such as BIR or ALT can occur in the absence of either pathway but not when both pathways or the Rad52 protein are inactivated (Le et al. 1999; Q. Chen et al. 2001). The two branches also differ in their involvement in several other cellular processes. For instance, MRX, unlike the RAD51 group, has been implicated in SSA, NHEJ, and intra-S-phase checkpoint activation (Haber 1998; D’Amours and Jackson 2001; Grenon et al. 2001).

Here we demonstrate another difference between the RAD51 and the RAD50 pathways: their effect in top3 mutants. In contrast to the Rad51 group proteins, the MRX complex becomes almost essential upon mutation of TOP3 (Figure 5A). The mrx mutants also show marked synthetic growth and HU/MMS resistance defects with mutation of SGS1 (Figure 5, B and C). Thus, MRX function is important when the Sgs1-Top3 pathway is on its own, but rad51Δ also partially rescues top3 slow growth. The RAD52 epistasis group has been genetically inactivated and almost essential when Sgs1 acts alone. MRX involvement in SSA and in intra-S-phase checkpoint activation may make it important in both sgs1 and top3 backgrounds. Because DNA metabolism defects are characteristic of both top3 and sgs1 mutants, checkpoints are particularly important in these mutants for activation of repair and recombination pathways. Presumably,

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**Figure 6.** Genetic interactions of top3 and sgs1 mutants with deletions of RAD59, RAD1, RDH54, and YKU70. (A) Deletion of TOP3 shows a synergistic growth defect with deletion of RDH54, which in turn is suppressed by deletion of SGS1. (B) Deletion of TOP3 does not show a synergistic defect with deletion of RAD59. (C) Deletion of TOP3 shows a synergistic growth defect with deletion of RAD1, which in turn is suppressed by deletion of SGS1. (D) Deletion of TOP3 does not show a synergistic defect with deletion of YKU70. (E) Deletion of RAD59, RAD1, or RDH54 increases HU sensitivity in an sgs1Δ background. HU sensitivity of the rad1 and rdh54 mutants at the concentration shown is identical to that of the wild-type strain and these controls are not shown. (F) Deletion of RAD59, RAD1, or RDH54 increases MMS sensitivity in an sgs1Δ background. rad1 and rdh54 strains do not exhibit sensitivity at these MMS concentrations and are indistinguishable from wild type in the graph.
such defects are more severe in top3 mutants, so checkpoints become essential. This idea is supported by the observation that mutation of the central S. cerevisiae checkpoint genes, MEC1 and RAD53, leads to lethality in a top3 background and to slow growth in an sgs1 background (Chakraverty et al. 2001; our unpublished results).

Roles of other recombination and repair genes in top3 mutants: Rdh54 and Rad1, neither of which is required to maintain a normal growth rate in an otherwise wild-type background, become critical for growth when Top3 is inactivated (Figure 6, A and C). Similarly, while neither Rdh54 nor Rad1 is important for repairing HU- or MMS-induced damage in wild-type cells (at least at the drug concentrations we tested), they become important for HU/MMS resistance in the absence of Sgs1 (Figure 6, E and F). The Rad1/Rad10 complex is thought to be involved in recombination processes that require removal of nonhomologous sequences from the ends of recombining DNA molecules (Fishman-Lobell and Haber 1992). top3 mutants may rely on such recombination processes for improved survival, while sgs1 mutants may utilize these pathways when confronted with HU- or MMS-induced damage. We have shown previously that genome rearrangement in top3 mutants is dependent on RAD1 (Bailis et al. 1992). The results presented here suggest that SSA, a RAD1-dependent process, provides an alternative to RAD51-dependent recombination in top3 mutants, especially in regions that contain direct repeats, such as SUP4 and rDNA.

The importance of Rdh54 in top3 and sgs1 backgrounds may reveal new functions of this protein in DNA metabolism in haploid cells. Previously, Rdh54 was shown to have diploid-specific roles in mitotic and meiotic recombination (Klein 1997; Shinohara et al. 2000). In haploid yeast, Rdh54 has been implicated in “adaptation,” a process during which a cell proceeds with mitosis in the presence of an unrepaired DSB after a cell cycle arrest in G2/M (Lee et al. 2001). This role of Rdh54 may be critical in top3 mutants, since “DNA damage” is constantly created by Sgs1 and the cell may need to “adapt” to it to divide. yKu70 has also been implicated in adaptation, yet deletion of YKU70 does not have a detrimental effect on growth in the absence of Top3 (Lee et al. 1998). This difference can be explained by proposing that Rdh54 and yKu70 detect different structures as signals of DNA damage and that yKu70 does not detect DNA intermediates generated by Sgs1. For example, yKu70 may detect DSBs, whereas Rdh54 recognizes other kinds of DNA structures. Alternatively, yKu70 may recognize damage mainly during G1, while Rdh54 is involved in damage recognition during S and/or G2 phases.

The results presented in this report are supported by other observations suggesting that when the Sgs1-Top3 pathway is impaired, Rad51-dependent recombination plays a detrimental role and cells rely on MRX- and Rad1-dependent processes for survival. For example, mutations of SGS1 or TOP3 exhibit synergistic growth defects with mutation of another helicase, Srs2 (Gangloff et al. 2000; McVey et al. 2001). Interestingly, mutation of several RAD51 epistasis group genes rescues sgs1 srs2 defects (Gangloff et al. 2000; McVey et al. 2001). sgs1 srs2 mutants also show several other genetic interactions similar to top3 mutants; they are inviable in combination with mutations in MRX genes and RAD1 but show no synthetic defect with a deletion of YKU70 (McVey et al. 2001).

Recombination at the SUP4 locus: While mutation of TOP3 or SGS1 leads to an overall hyper-recombination phenotype at SUP4, both GC and SSA events are observed in these mutants in proportions roughly similar to the wild-type strain. This is consistent with previous observations where mutation of SGS1 did not affect GC rates (Watt et al. 1996). Interestingly, at SUP4-o, the fraction of GCs with crossing over (classes III and IV) is increased in top3 mutants relative to wild type. This may indicate that the absence of Top3 affects resolution of recombination intermediates, leading to increased formation of crossover events. Alternatively, the recombinogenic structures created by Sgs1 and left unresolved by Top3 may be preferentially channeled into recombination pathways that result in crossover products.

Although mutation of RAD51, -54, -55, or -57 leads to a slight increase in overall deletion formation at SUP4-o, no GC events were observed in these mutants. We interpret this to mean that these proteins are required for GC at this locus, and that, in these mutants, recombinogenic lesions are channeled into RAD51-independent, rearrangement-prone recombination events, such as SSA, BIR, or NHEJ. However, unless extensive degradation of DNA ends takes place prior to end joining, NHEJ would not result in large deletions and therefore would not be detected in our assay. Deletion of TOP3 in a rad51, -54, -55, or -57 background further increases SUP4-o deletion rates in these mutants 12- to 20-fold. This increase is less severe than the effect that a top3 mutation has in a Rad+ strain, where SUP4 recombination is increased 90-fold. These results suggest that in top3 mutants, in the absence of the Rad51 pathway, Sgs1 creates fewer recombinogenic intermediates. Such intermediates are consequently processed by RAD51-independent mechanisms, leading to an increase in SSA classes and to an abolition of GC classes at SUP4.

Sgs1-Top3 and recombination during DNA replication: We observe that in mutants of the RAD51 epistasis group, the ability of Sgs1 to cause slow growth and hyper-recombination in the absence of Top3 is diminished. This observation can be explained by a model in which the homologous recombination machinery helps recruit the Sgs1-Top3 complex to its site of action via the Rad51-Sgs1 interaction (Figure 7). In top3 mutants, Sgs1 molecules create intermediates that are channeled into RAD51-dependent (GC) and RAD51-independent
(SSA) recombination pathways, in proportions similar to those in wild-type cells (Table 3). These intermediates are also responsible for the slow growth of top3 mutants, since inactivation of SGS1 fully rescues the slow growth phenotype. Mutation of the homologous recombination complex results in decreased localization of Sgs1 to chromosomal sites and/or in decreased ability of Sgs1 to create these detrimental structures. Thus, fewer intermediates that require resolution by Top3 are created and growth is improved. The lesions that Sgs1 does create in these rad top3 mutants are channeled into RAD51-independent recombination pathways, such as SSA.

In an alternative model, Sgs1 creates a substrate for Top3 that, when left unresolved in top3 mutants, is channeled into various recombination pathways for resolution. The Sgs1-Rad51 physical interaction may help channel this intermediate into the Rad51 recombination pathway. The processing of the substrate by the homologous recombination machinery may create detrimental chromosomal structures that cause slow growth and other defects seen in top3 mutants, while its alternative processing (e.g., by Rad1-dependent SSA) may be beneficial. Further biochemical and cell biological studies are necessary to distinguish between the two models.

Both scenarios presented above are consistent with the idea that the Sgs1-Rad51 interaction is important for creation of the detrimental intermediate in top3 mutants. A compelling piece of evidence in favor of this notion comes from studies of the in vivo roles of different domains of Sgs1. The C-terminal 200 amino acids that mediate Sgs1 interaction with Rad51 are dispensable for Sgs1 function in an otherwise wild-type background (Mullen et al. 2000; Wu et al. 2001). However, in a top3 background, the sgs1-ΔC200 allele behaves similarly to sgs1Δ, rescuing the slow growth caused by mutation of TOP3 (Mullen et al. 2000). These results suggest that the Sgs1-Rad51 interaction is important for Sgs1 to create chromosomal intermediates that require resolution by Top3.

Other studies have suggested a relationship between Sgs1 and homologous recombination. A model has been proposed in which Sgs1 and Srs2 regulate the processing of recombination intermediates during DNA replication (Gangloff et al. 2000). This model is supported by the observation that Sgs1 physically interacts with Rad51 via the Sgs1 C terminus (Wu et al. 2001). To investigate further the relationship between Sgs1 and the homologous recombination complex, we tested the sensi-
tivity of the sgs1 and sgs1 rad mutants to HU and MMS. Here we show that SGS1 is not in the RAD52 epistasis group for HU or MMS resistance, suggesting that the Sgs1/Top3 complex and the Rad proteins have independent roles in protecting the genome against damage induced by these agents (Figure 3). However, these results do not contradict the hypothesis that Sgs1/Top3 and the homologous recombination complex interact during DNA replication to promote genome integrity. The data we present in this report are supported by other observations that homologous recombination plays a detrimental role in cells that lack the activity of either a RecQ homolog or a topoisomerase III. For example, in budding yeast, blocking meiotic recombination rescues the sporulation defect of top3 homozygous diploids (Gangloff et al. 1999). Additionally, as discussed above, sgs1 snr2 mutants are rescued by inactivating the RAD51 pathway (Gangloff et al. 2000). Moreover, in humans, hyper-recombination between sister chromatids is a major contributor to the genomic instability characteristic of BLM cells, while aberrant recombination intermediates are found in WRN cells (Geman 1993; Prince et al. 2001). Further studies of the Sgs1-Top3 complex and its homologs promise to yield new information about its cellular role, the defects associated with its loss, and its relationship to homologous recombination.

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LITERATURE CITED


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