Requirement for Three Novel Protein Complexes in the Absence of the Sgs1 DNA Helicase in *Saccharomyces cerevisiae*

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ABSTRACT

The *Saccharomyces cerevisiae* Sgs1 protein is a member of the RecQ family of DNA helicases and is required for genome stability, but not cell viability. To identify proteins that function in the absence of Sgs1, a synthetic-lethal screen was performed. We obtained mutations in six complementation groups that we refer to as SLX genes. Most of the SLX genes encode uncharacterized open reading frames that are conserved in other species. None of these genes is required for viability and all SLX null mutations are synthetically lethal with mutations in TOP3, encoding the SGS1-interacting DNA topoisomerase. Analysis of the null mutants identified a pair of genes in each of three phenotypic classes. Mutations in MMS4 (SLX2) and SLX3 generate identical phenotypes, including weak UV and strong MMS hypersensitivity, complete loss of sporulation, and synthetic growth defects with mutations in TOP1. Mms4 and Ssk3 proteins coimmunoprecipitate from cell extracts, suggesting that they function in a complex. Mutations in SLX5 and SLX8 generate hydroxyurea sensitivity, reduced sporulation efficiency, and a slow-growth phenotype characterized by heterogeneous colony morphology. The Ssk5 and Ssk8 proteins contain RING finger domains and coimmunoprecipitate from cell extracts. The SLX1 and SLX4 genes are required for viability in the presence of an sgs1 temperature-sensitive allele at the restrictive temperature and Ssk1 and Ssk4 proteins are similarly associated in cell extracts. We propose that the MMS4/SLEX3, SLX5/8, and SLX1/4 gene pairs encode heterodimeric complexes and speculate that these complexes are required to resolve recombination intermediates that arise in response to DNA damage, during meiosis, and in the absence of SGS1/TOP3.

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*Sgs1* is a member of the RecQ family of DNA helicases, which includes RecQ from *Escherichia coli* (Umezu and Nakayama 1993), Rqh1 from *Schizosaccharomyces pombe* (Murray et al. 1997; Stewart et al. 1997), and BLM (Ellis et al. 1995), WRN (Yu et al. 1996), and RecQL4 from humans (Kitao et al. 1999). These proteins have been shown to possess 3’ to 5’ DNA helicase activity (Lu et al. 1996; Gray et al. 1997; Karow et al. 1997; Bennett et al. 1998; Umezu et al. 1999) and mutations in the human genes give rise to diseases that share a common predisposition to cancer owing to defects in genome stability. Bloom’s syndrome cells (BLM) show decreased DNA strand breaks, increased rates of sister chromatid exchange, increased DNA repair, and sensitivity to the DNA-damaging agent 4-NQO (Fujikawa et al. 1977; Salk et al. 1981; Gehbhart et al. 1988). Rothmund-Thomson syndrome fibroblasts (RecQL4) show elevated rates of chromosomal breaks and rearrangements (Miozzo et al. 1998).

Mutations in *SGS1* mirror the chromosomal instabilities of these human diseases by generating increased rates of mitotic recombination, both at the ribosomal DNA (rDNA) locus and throughout the genome (Gangloff et al. 1994; Watt et al. 1996), and increased rates of chromosome loss and missegregation (Watt et al. 1995). Hyper-recombination at the rDNA locus in sgs1 mutants has been proposed to accelerate the production of extrachromosomal rDNA circles leading to premature aging in these cells (Sinclair and Guarente 1997; Sinclair et al. 1997). The nucleoli of cells lacking the Sgs1 protein become enlarged and fragmented (Sinclair et al. 1997), presumably due to increased production of rDNA circles (Sinclair and Guarente 1997). Mutations in *SGS1* also result in hypersensitivity to the DNA-damaging agent methyl methanesulfonate (MMS; Mullen et al. 2000) and hydroxyurea (HU; Yamagata et al. 1998), suggesting that Sgs1 plays a role in DNA repair (Chakraverty and Hickson 1999). Diploids lacking Sgs1 show reduced sporulation efficiency and reduced spore viability, indicating that the protein is involved in meiosis (Gangloff et al. 1999).

Mutations in *SGS1* were first identified on the basis of their ability to suppress the slow-growth phenotype of cells with a mutation in the type I DNA topoisomerase TOP3 (Gangloff et al. 1994). *SGS1* and *TOP3* interact...
in a two-hybrid assay, and epistasis experiments indicate that SGS1 functions upstream of TOP3 (Gangloff et al. 1994). This suggests that the Sgs1 DNA helicase creates a toxic substrate that is normally processed by Top3. Enzymatically, Top3 resembles DNA topoisomerase III a toxic substrate that is normally processed by Top3. (Kim and Wang 1992). Taken together with the ability of E. coli topoisomerase III to decatenate gapped ssDNA (DiGate and Marians 1988), it has been proposed that Sgs1 and Top3 might function at the termination of DNA replication to decatenate gapped daughter molecules (Wang 1991; Rothstein and Gangloff 1995). The idea that Sgs1 provides the ssDNA substrate for Top3 is supported by the recent demonstration that E. coli RecQ stimulates the ability of topoisomerase III to decatenate gapped ssDNA (Harmon et al. 1999). On the other hand, an interaction between Sgs1 and Top2 was identified by the two-hybrid system, suggesting that SGS1 functions in the TOP2 pathway for chromosomal segregation (Watt et al. 1995).

Other models of SGS1 function have considered the in vivo role of bacterial recQ. In E. coli, recQ functions in the recF recombination pathway, which is required to resume synthesis at stalled DNA replication forks (Courcelle et al. 1997). Similarly, Sgs1 might play a role in restarting stalled replication forks, which could explain both the hypersensitivity of sgs1 mutants to MMS and HU (Chakraverty and Hickson 1999) and the failure of S. pombe rqh1 mutants to recover from HU arrest (Stewart et al. 1997). Support for this idea is provided by the finding that BLM protein binds Holliday junctions and promotes branch migration in vivo (Karow et al. 2000). Still unexplained is whether Top3 plays a role in this process. Also unexplained is the genetic interaction of SGS1 mutants with mutations in TOP1 (encoding another type I topoisomerase) such that the sgs1 top1 double mutant grows more slowly than either single mutant (Lu et al. 1996).

To help define the primary role of SGS1, yeast cells were screened for mutations that are lethal in the absence of SGS1. This screen identified six novel open reading frames of unknown function that we refer to as SLX genes (synthetic lethal of unknown function). A detailed analysis of SLX2 (MMS4) and SLX3 revealed that null mutants produce identical phenotypes and function in the same genetic pathway in response to DNA damage. We show that the remaining four SLX genes fall into two additional phenotypic classes: SLX5/8 and SLX1/4. The simplest model suggests that the SLX proteins act in pairs, or heterodimers, to perform three different functions in the absence of Sgs1. This model is supported by immunoprecipitation experiments that identified three specific complexes of SLX proteins. We interpret these results on the basis of current models of Sgs1/Top3 function and propose that the three SLX complexes play a role in the resolution of recombination intermediates generated in the absence of either SGS1 or TOP3.

**Yeast strains and growth conditions:** Yeast strains are listed in Table 1. Strain construction, growth, and transformation followed standard protocols (Rose et al. 1990).

**Plasmid construction:** pJM500 was constructed by ligating a BamHI/SalI ADE3 fragment and a Xhol/Sacl SGS1 PCR product into BamHI/Sacl-cut pRS416 (Sikorski and Hieter 1989). pJM501 was constructed by ligating the Xhol/Sacl SGS1 PCR product into Xhol/Sacl-cut pRS413. Plasmids pJM555 and pJM6710 were described previously (Mullen et al. 2000), as were pJM31 and pJM37 (Lu et al. 1996).

**Isolation of synthetic lethal mutants:** [MY329a and MY329a both carry pJM500, which contains the SGS1, ADE3, and URA3 genes. When the plasmid is lost, the colony color changes from red (ade2) to white (ade2 ade3). JMY329 and JMY332 were grown in synthetic complete media lacking uracil to ~2 × 106 cells/ml, washed, and concentrated to ~8 × 106 cells/ml in 100 mM NaPO4, pH 7. Strains were mutagenized by the addition of 50 μl of ethyl methanesulfonate (EMS; Sigma, St. Louis) to 1.7 ml of cells, shaking gently at 30°C for 1 h. One hundred-microliter aliquots were removed at 0, 15, 30, 45, and 60 min after EMS addition, neutralized with 4 ml of 5% sodium thiosulfate, diluted, and plated on YPD (yeast extract/peptone/dextrose) plates to determine the killing rate. One hour in EMS killed 64% of JMY332 cells and 69% of MY329 cells. Cells from this time point were plated and screened for loss of sectoring. Nonsectoring colonies were replica plated onto synthetic complete plates containing 5-fluoroorotic acid (FOA), which allows growth of Ura- cells only, and any that grew were discarded (Boeke et al. 1987). To eliminate strains that had integrated the plasmid, FOA sensitive (FOA2) strains were transformed with pJM501 (SGS1-HIS3) and restreaked onto FOA. Strains that became FOA resistant (FOA3) with pJM501 were designated synthetic lethal strains. To place the mutations into complementation groups (CGs), all synthetic lethals from JMY329 were mated to all from JMY332 and streaked onto YPD plates (to score sectoring) and FOA plates (to score growth). Of ~22,000 JMY329 colonies screened, 19 synthetic lethal strains were isolated, 18 of which have been placed into CGs. Of ~19,000 JMY332 colonies screened, 16 synthetic lethal strains were isolated, all of which have been placed into CGs.

**Cloning the SLX genes:** To clone the SLX genes, a leu2 strain from each complementation group was transformed with a LEU2 genomic library on the basis of either Yep13 or the CEN plasmid p366. Approximately 6000–8000 transformants (per complementation group) were replica plated onto FOA plates and screened for growth. Complementing plasmids were rescued and transformed back into the starting strain, another member of that CG (when possible), and a member of a different CG. Plasmids that complemented only the original CG were sequenced from either end to identify open reading frames (ORFs). Plasmids complementing both CGs were assumed to contain SGS1. All complementing plasmids contained multiple ORFs, so individual ORFs were cloned by PCR into pRS415 (Sikorski and Hieter 1989), transformed into the mutants, and tested for growth on FOA and sectoring on YPD. For each SLX gene, one specific ORF was shown to complement the synthetic-lethal phenotype. Each complementing ORF was shown to be genetically linked to the SLX
TABLE 1

S. cerevisiae strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference or source</th>
</tr>
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<tbody>
<tr>
<td>W303-1a</td>
<td>MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</td>
<td>Thomas and Rothstein (1989)</td>
</tr>
<tr>
<td>RS190</td>
<td>W303-1a top1-8::LEU2</td>
<td>Brill and Sternglanz (1988)</td>
</tr>
<tr>
<td>CHY128</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 leu2-3,112 lys2 can1-100</td>
<td>Chris Hardy</td>
</tr>
<tr>
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<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</td>
<td>Mullen et al. (2000)</td>
</tr>
<tr>
<td>HKY715</td>
<td>MATa ura3-1 leu2-3,112 his1-15 top1-1 hisG srs2::TRP1 can1::hisG</td>
<td>Hannah Klein</td>
</tr>
<tr>
<td>JMY329</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 lys2 can1-100 sgs1-3::TRP1 + pJM500 (SGS1/URA3/ADE3)</td>
<td>This study</td>
</tr>
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<td>JMY331</td>
<td>W303-1a sgs1-3::TRP1 top1-8::LEU2</td>
<td>This study</td>
</tr>
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<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-3::TRP1 + pJM500 (SGS1/URA3/ADE3)</td>
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<tr>
<td>JMY361</td>
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</tr>
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<td>This study</td>
</tr>
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<td>W303-1a sgs1-3::TRP1</td>
<td>This study</td>
</tr>
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<td>JMY380</td>
<td>W303-1a sgs1-3::TRP1</td>
<td>This study</td>
</tr>
<tr>
<td>JMY422</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 slx2-10::KAN sgs1-3::TRP1 + pJM500 (SGS1/URA3/ADE3)</td>
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<td>JMY503</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-20::loxP top1-11::loxP leu2-3,112 can1-100 sgs1-3::TRP1 + pJM500 (SGS1/URA3/ADE3)</td>
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<td>JMY506</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-31::loxP top1-11::loxP leu2-3,112 can1-100 sgs1-3::TRP1 + pJM500 (SGS1/URA3/ADE3)</td>
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<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-1::loxP top1-11::loxP sgs1-1::loxP top1-11::loxP + pJM6401 (SLX4/URA3/ADE3)</td>
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<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-1::loxP top1-11::loxP + pJM500 (SLX4/URA3/ADE3)</td>
<td>This study</td>
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<td>JMY612</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-1::loxP top1-11::loxP + pJM500 (SLX4/URA3/ADE3)</td>
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<tr>
<td>JMY613</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-1::loxP top1-11::loxP + pJM500 (SLX4/URA3/ADE3)</td>
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<td>W303-1a sgs1-3::TRP1</td>
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<td>JNY1061</td>
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<td>JNY1116</td>
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<td>JNY1118</td>
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<td>JMY1221</td>
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<td>JMY1222</td>
<td>MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-3::TRP1 + pJM500 (TOP3/URA3/ADE3)</td>
<td>This study</td>
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</tbody>
</table>

locus as follows. The wild-type SLX ORF in an sgs1Δ mutant was marked (but not disrupted) with HIS3 by integrative transformation, crossed to the respective mutant (sgs1Δ slx his3 + pJM500), and the diploid was subjected to tetrad analysis. Linkage analysis results were as follows: SLX1: (51 spores) 35 FOAΔ-His+, 16 FOAΔ-His-, 1 FOAΔ-His+. SLX2: (37 spores) 21 FOAΔ-His+, 10 FOAΔ-His-, 6 FOAΔ-His+. SLX3: (46 spores) 26 FOAΔ-His+, 20 FOAΔ-His-. SLX4: (26 spores) 17 FOAΔ-His+, 9 FOAΔ-His-. SLX5: (34 spores) 18 FOAΔ-His+, 16 FOAΔ-His-.

SLXDeletions: Two alleles of SLX1 were made: slx1-10::TRP1 and slx1-11::HIS3. To create slx1-11::HIS3, a BglII site was intro-
duced after amino acid 30 of SLX1 by PCR and a HIS3 fragment was ligated into the BglII/XhoI-cut SLX1 gene (XhoI cuts at amino acid 274 out of 304). To create slx1-10::TRP1, a BamHI/XhoI TRP1 fragment was cloned into the same BglII/XhoI-cut SLX1 gene. SLX2, SLX3, SLX4, and SLX8 were all disrupted by PCR-mediated kanamycin disruption (WACH et al. 1994), and each ORF was removed completely. The slx5-10::TRP1 null allele was made by PCR-mediated disruption with TRP1, removing the entire ORF.

**Epitope tagging:** The SLXI gene and a fragment encoding a C-terminal myc tag (LONGTINE et al. 1998) was placed under the GAL1 promoter in plasmid pRS423 to create pNj6120 (2μ, HIS3, GAL-SLXI-13MYC). The MMS4 gene and a fragment encoding a C-terminal triple-hemagglutinin (HA) tag was placed under the GAL/Phosphoglucomutase (PGK) promoter of plasmid pHG2 (2μ, LEU2-d, GAL-PGK-His) to create pNj6209 (2μ, LEU2-d, GAL-PGK-6His-MMS4-3HA). A multicopy URA3 vector expressing a galactose-inducible SLX3 with C-terminal V5 and hexa-histidine tags was obtained from Invitrogen (pYDR386WY; 2μ, URA3, GAL-SLX3-V5-6His). The SLX5 gene was subcloned into plasmid pNj6209 to create pNj6409 (2μ, LEU2-d, GAL-PGK-6His-SLX3-3HA). The SLX5 gene and a fragment encoding a C-terminal V5-hexa-histidine tag were placed under the control of the GAL1 promoter in vector pRS424 to create pNj6508 (2μ, HIS3, GAL-SLX5-V5-6His). The SLX8 gene was subcloned into plasmid pNj6209 to create pNj6806 (2μ, LEU2-d, GAL-PGK-6His-SLX8-3HA).

**Galactose to glucose shift:** JMY332 and JMY422 carrying pJM6710 (GAL-SGS1-LEU2) were grown in yeast/peptone/galactose liquid (YPGal) at 30°C to ~1 × 10^9 cells/ml and glucose was added to a final concentration of 2%. Aliquots were removed at various time points after the addition of glucose and fixed in 0.37% formaldehyde/phosphate-buffered saline (PBS) for 20 min at room temperature. Cells were then washed three times in PBS, stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) for 12 min at room temperature, rinsed three times with PBS, and examined by DIC and fluorescence microscopy.

**Immunoprecipitations:** Yeast cells were grown to an OD_{600} of 1.0 in the appropriate selective medium containing 2% each of raffinose and sucrose. Expression was induced by the addition of galactose (2% final concentration) and cells were incubated for 3 hr at 30°C. Extracts were prepared and proteins were immunoprecipitated and immunoblotted as described (MULLEN et al. 2000). Approximately 200 μg of crude extract was mixed with an equal volume of RIPA buffer (HARLOW and LANE 1988) and incubated on ice for 1 hr with 5 μg (anti-HA, anti-myc; Boehringer Mannheim, Indianapolis) or 1 μg (anti-V5; Invitrogen, Carlsbad, CA) monoclonal antibody. Following immunoprecipitation, the beads were washed three times in RIPA buffer prior to immunoblotting.

**RESULTS**

**An SGS1 synthetic-lethal screen:** The colony color assay of Bender and Pringle was used to identify mutants that require SGS1 for viability (BENDER and PRINGLE 1991). An sgsΔ ade2 ade3 strain was constructed and transformed with pJM500, which contains the SGS1, ADE3, and URA3 genes. Since ade2 strains are red and ade2 ade3 strains are white, the transformed strain acquires a red color (ade2 ADE3). When grown without selection pJM500 is lost and the strain’s color changes from red (ade2) to white (ade2 ade3), with colonies exhibiting a sectoring morphology. Following mutagenesis we screened for nonsectoring colonies that retain the plasmid in the absence of selection. Of 41,000 mutagenized colonies that were screened, 35 potential sgsΔ mutants were identified. Of these mutants, 34 were placed into seven complementation groups, as shown in Table 2. Wild-type (wt) copies of the genes mutated in these groups were cloned by complementation with plasmid libraries (MATERIALS AND METHODS). Complete sgsΔ deletions were created and all were found to be viable in the wt SGS1 background (Table 2). Six of the seven sgsΔ null mutations were inviable in combination with sgs1Δ (Table 2) and are considered authentic synthetic-lethal mutations. During the course of this work SLX2 was independently identified as MMS4 (XIAO et al. 1998).

Consequently, we hereafter refer to SLX2 as MMS4.

The synthetic lethal phenotype is shown in the top two panels of Figure 1. For simplicity, only an mms4Δ sgs1Δ strain is presented, but all of the double mutants behave identically in this regard (Figure 1 and data not shown). An mmsΔ sgs1Δ double mutant carrying pJM500 (SGS1/URA3/ADE3) was transformed with a LEU2 vector containing no insert, wild-type SGS1, sgs1-hd (containing a helicase-inactivating point mutation), or SGS1 conditionally expressed under the GAL1 promoter. When these strains were streaked onto galactose media containing the drug FOA to select against pJM500, only the SGS1 plasmid and the GAL-driven SGS1 plasmid allowed growth (Figure 1, top left). On FOA-glucose, only SGS1 under its natural promoter allows growth, confirming that Sgs1 expression is absolutely required for viability in the double mutant (Figure 1, top right). The sgs1-hd plasmid cannot support growth on either glucose or galactose, indicating that the helicase activity of Sgs1 is necessary for the survival of the double mutant. All of the sgsΔ mutants require the Sgs1 protein and, specifically, the helicase activity of Sgs1 to survive (Table 2).

We examined the mmsΔ sgs1Δ cells carrying the GAL-SGS1 plasmid after 2 hr in glucose to determine the immediate effect of glucose repression of SGS1. In glucose, the double-mutant cells swell and many have large buds with diffusely staining DNA in the neck between mother and daughter (Figure 1, arrows in bottom right). The sgs1Δ single-mutant cells carrying the same plasmid are unaffected by the glucose shift and any large-budded cells have two separate nuclei (Figure 1, middle right). While the effects of the glucose shift can be seen after only 2 hr, loss of viability does not begin until ~6–8 hr (data not shown). At 18 hr, the cells are greatly swollen, consisting of both large-budded and single cells with very diffusely staining DNA (data not shown). Since the cell number continues to increase in glucose, we interpret the 2-hr phenotype to be a delay in the late S/G2 phase of the cell cycle. This phenotype is similar to the delay observed in exponentially growing top3 mutants (GANGLOFF et al. 1994).

**Specificity of the SLX/SGS1 interactions:** The 1447-amino-acid (aa) Sgs1 protein has two functional domains consisting of the N-terminal domain (aa 1–652)
Genes Required in the Absence of SGS1

TABLE 2

SGS1 synthetic-lethal mutants

<table>
<thead>
<tr>
<th>Complementation group</th>
<th>No. of alleles</th>
<th>Mutant gene</th>
<th>Complementation with sgs1 alleles*</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SGS1</td>
</tr>
<tr>
<td>SLX1</td>
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<td>SLX8</td>
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<td>YER116C</td>
<td>+</td>
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</table>

a slxΔ sgs1Δ complementation by the indicated SGS1 allele: +, viable; –, lethal; ND, not done.

b srs2Δ sgs1Δ strains are viable but slow growing.

and the DNA helicase domain (aa 653–1447). Both domains are required for wild-type Sgs1 activity, although the C-terminal 200 amino acids are dispensable for most Sgs1 activities (Mullen et al. 2000). To determine which of the functional domains was required for viability in the slx backgrounds, we tested whether the amino- and carboxy-terminal truncation alleles of SGS1 could rescue the slxΔ sgs1Δ mutants. The double mutants carrying pJM500 were transformed with a LEU2 plasmid containing a deletion allele of SGS1 and then streaked onto FOA to select for loss of pJM500. None of the N-terminal SGS1 deletions, including a small 158-aa deletion, could rescue any of the double mutants that were tested (Table 2). Only the smallest C-terminal deletion of 200 amino acids of SGS1 rescued the double mutants, while deletion of 300 amino acids was lethal (Table 2; Mullen et al. 2000). These results demonstrate that the slxΔ mutations behave similarly to one another and require both functional domains of Sgs1 to maintain viability.

To further test the specificity of the interactions between SGS1 and the SLX genes, slxΔ mutations were introduced into a number of mutant DNA helicase backgrounds by genetic crosses. Tetrad analysis revealed that the mutations rad25-Xp (the yeast homolog of XPBC/ERCC3 helicase, Park et al. 1992; Guzder et al. 1994), pif1::TRP1 (a mitochondrial and nuclear DNA helicase, Lahaye et al. 1993; Schulz and Zakian 1994), dna2-1p (a putative replicative DNA helicase in yeast, Budd et al. 1995), or srs2::TRP1 (a DNA repair helicase, Rong and Klein 1993; Rong et al. 1991) do not cause synthetic lethality in conjunction with mms4Δ or slx3Δ. In fact, these double mutants showed no obvious growth defects (data not shown). Thus, the SLX genes are synthetically lethal only with the SGS1 DNA helicase, and not with four other DNA helicases.

SRS2 is not essential in the absence of SGS1: One of the genes isolated in our screen was SRS2/HPR5, a DNA helicase that, when mutant, suppresses rad6 and rad18 UV hypersensitivity and causes a hyper-recombination phenotype (Rong and Klein 1993; Rong et al. 1991). However, when an srs2 null was crossed to an sgs1 null background, the sgs1Δ srs2Δ double mutant was viable, demonstrating that SRS2 is not essential in the absence of SGS1.
and sporulated, we obtained complete tetrads (Figure 2A). While some of the double-mutant spores failed to grow, at least half of them survived, albeit with a slow-growth phenotype (Figure 2A, arrows). Although srs2Δ sgs1Δ double mutants were reported to be synthetically lethal (Lee et al. 1999), our results indicate that they are synthetically sick as observed by others (Gangloff et al. 2000). It seems likely that srs2 was detected in the synthetic lethal screen because of additional mutations in the highly mutagenized original strain. While we cannot rule out the possibility of a suppressor in our strain background, further genetic analysis revealed that SRS2 is distinct from authentic SLX genes (see below and Figure 2C).

The SLX genes are essential in the absence of TOP3: Each sbsΔ mutant was crossed to a top3Δ strain and the resulting tetrads were analyzed. In all cases, no viable double-mutant spores were found, indicating that these mutations are synthetically lethal with top3 (Figure 2B and data not shown). To confirm that these genes were not required simply for germination, strains were constructed that were double mutant for top3Δ and each of the sbsΔ mutations and complemented with a TOP3/URA3 plasmid. These strains failed to grow when streaked onto FOA, confirming that all of the SLX genes are essential in the absence of TOP3 (Figure 2C). Since SGS1 and TOP3 show an epistasis relationship and interact by two-hybrid assay (Gangloff et al. 1994), these results strongly suggest that the SLX genes function in one or more pathways parallel to that of SGS1/TOP3. Consistent with this conclusion, adding the sgs1Δ mutation to mms4Δ top3Δ, sxs3Δ top3Δ, or sxs4Δ top3Δ double mutants did not suppress lethality (Figure 2C). In contrast, adding the sgsΔ mutation to the top3Δ srsΔ double mutant suppressed the lethality and allowed the triple mutants to live (Figure 2C). We conclude that SRS2 interacts with TOP3 and SGS1 in a manner distinct from the interactions of authentic SLX genes.

Sensitivity to MMS and HU identify three phenotypic classes of SLX mutants: We tested whether the sbsΔ strains had any phenotypes in common with sgsΔ strains. Wild-type yeast cells are able to grow in the presence of methyl methansulfonate (MMS) and hydroxyurea (HU), whereas sgs1Δ strains are hypersensitive to these drugs and grow poorly in their presence (Yamagata et al. 1998; Mullen et al. 2000). To test the sbsΔ strains for MMS hypersensitivity, wild-type and mutant strains were serially diluted and replica plated onto MMS and control plates. After 2 days at 30°C, the mms4Δ and sxs3Δ strains grew very poorly on the MMS plate (Figure 3, left). On the basis of this assay, the mms4Δ and sxs3Δ strains are about as sensitive to MMS as is the sgs1Δ strain. This phenotype is not true of all sbsΔ mutants, as the other strains are relatively insensitive to MMS and grow as well, or almost as well, as the wild-type parent. These findings suggest that Mms4 and Slx3, like Sgs1, are involved in repairing or responding to the DNA damage caused by MMS.

The sbsΔ strains were similarly tested for HU sensitivity. In the presence of HU the growth of sxs5Δ and sxs8Δ strains was strongly inhibited, as was the growth of the sgs1Δ strain (Figure 3, middle). In contrast, the growth of mms4Δ and sxs3Δ mutants was only weakly inhibited, while the growth of sxs1Δ and sxs4Δ strains was indistinguishable from wild type. These findings suggest that Slx5 and Slx8, and perhaps Slx2 and Slx3, are involved in the response to DNA synthesis arrest caused by HU.

We next tested whether mms4Δ and sxs3Δ strains were hypersensitive to UV radiation and if they acted in the same genetic pathway. Cells were grown to log phase, diluted, and plated onto YPD. Duplicate plates were subjected to increasing amounts of UV radiation, incubated at 30°C for 2 days, and colonies were counted. Two known UV-sensitive strains, rad3Δ and rad9Δ, were included as controls. The mms4Δ and sxs3Δ strains showed identical profiles of weak UV sensitivity. At the highest UV dose, the mms4Δ and sxs3Δ strains were ~40 times more sensitive than the wild-type parent and ~20 times less
sensitive than the rad9 mutant (Figure 4A). The mms4Δ slx3Δ double mutant had a UV sensitivity profile that was indistinguishable from either single mutant.

To measure more accurately the degree of MMS sensitivity shown by the mms4Δ and slx3Δ mutants, assays in liquid culture were performed. After 1 hr in 0.3% MMS, both the mms4Δ and slx3Δ mutants were ~50 times more sensitive than their isogenic parent (Figure 4B). Once again, the mms4Δ slx3Δ double mutant was not significantly more sensitive to MMS than was either single mutant (Figure 4B). Because the single and double mutants responded similarly to DNA damage caused by either UV radiation or MMS, we conclude that MMS4 and SLX3 function in the same genetic pathway.

**MMS4 and SLX3 share additional phenotypes:** The sgs1 mutation interacts with top1 such that the double mutant grows more slowly than either single mutant strain (Lu et al. 1996). To uncover interactions between the SLX genes and DNA topoisomerases, the slxΔ mutants were crossed to a top1Δ top2-1(ts) strain and the resulting tetrad was analyzed. Although the slxΔ mutation was unaffected by the addition of either top1Δ or top2-1(ts) (Table 3), we found that both mms4Δ and slx3Δ interacted genetically with mutations in TOP1 and TOP2. The mms4Δ top1Δ and slx3Δ top1Δ strains grew as slowly as the sgs1Δ top1Δ double mutant and more slowly than either single mutant (Figure 5). In addition, both mms4Δ top2-1 and slx3Δ top2-1 strains grew more slowly than single mutants at the semipermissive temperature of 30°C (data not shown).

Diploid strains homozygous for each of the slxΔ mutations were constructed and tested for their ability to sporulate. Diploids that were homozygous mutant for slx1Δ and slx2Δ were able to sporulate like wild type, but mms4Δ and slx3Δ homozygous diploids were completely defective and formed no complete ascis (Table 3). Interestingly, mms4Δ and slx3Δ diploids gave rise to rare two-spored ascis at nearly the same frequency. To determine whether the sporulation defect was in meiosis or in spore formation, the sporulated cultures were stained with DAPI to visualize the nuclei. If the cells were defective in spore formation but meiosis had occurred, some percentage of the cells would have two or four nuclei. Conversely, if the defect was in meiosis, all of the unsporulated cells would contain only one nucleus, which was what we observed (data not shown). Our results suggest that the mms4Δ and slx3Δ diploids are defective in meiosis and not spore formation. Additional tests on the mms4Δ and slx3Δ strains showed no significant effect on telomere length, heat shock sensitivity, growth at 37°C, or spontaneous mutation rate.

**MMS4/SLX3 sequence and predicted function:** MMS4 encodes a 691-amino-acid protein with a predicted molecular weight of 79 kD (Xiao et al. 1998). A BLAST analysis using the Mms4 sequence detected a potential homolog in Neurospora crassa (21% identical; 41% similar) that shows weak sequence similarity throughout its length (Figure 6A and data not shown). The SLX3 gene encodes a 632-amino-acid protein with a predicted molecular weight of 72 kD. BLAST analysis using Slx3 identified potential homologs in most highly sequenced organisms, including S. pombe, Drosophila melanogaster, Caenorhabditis elegans, Arabidopsis thaliana, and Homo sapiens (Figure 6B). These proteins range in size from 445 to 741 aa, with the greatest similarity localized to two regions near the C terminus. For example, while the two yeast proteins are 30% identical and 40% similar overall, they share 53% identity and 65% similarity over the region 380–446. These results suggest that MMS4 and SLX3 may be conserved in all eukaryotic species.

A BLAST analysis using the most highly conserved region of Slx3 identified a similar domain in the C terminus of the Rad1 protein of Saccharomyces cerevisiae (Figure 6B). Rad1 is a member of the heterodimeric Rad1/Rad10 endonuclease required for nucleotide excision repair (Bardwell et al. 1994) and normal levels of mitotic recombination (Schiestl and Prakash 1988;
Figure 5.—MMS4 and SLX3 interact with TOP1. The indicated single- and double-mutant strains were streaked onto YPD and grown at 30° for 2 days. As previously shown, the sgs1 top1 double mutant (JMY331) grows more slowly than either the top1 (RS190) or sgs1 (not shown) single mutants. The mms4 top1 (JMY442) and slx3 top1 (JMY446) double mutants grow more slowly and form smaller colonies than the top1 (RS190), mms4 (JMY375), and slx3 (JMY380) single mutants.

Figure 4.—Mutations in MMS4 and SLX3 are epistatic in response to DNA damage. (A) Exponentially growing cells were diluted appropriately in water, plated, exposed to increasing amounts of UV radiation, and incubated at 30° for 3 days. Viable colonies were counted and expressed as percentage viability of the untreated sample. (B) To overnight cultures growing at 30°, MMS was added to a final concentration of 0.3% and aliquots were removed at 15-min intervals. Neutralized samples were plated and viability was determined as described above.
these genes function in the same genetic pathway. Diploids homzygous for either slx5Δ or slx8Δ were tested for sporulation efficiency. In contrast to mms4Δ and slx3Δ mutants, slx5 and slx8 diploids were able to sporulate at a reduced frequency, ~30% of the wild-type level (Table 3). These results indicate that SLX5 and SLX8 are required for efficient sporulation and that they function in the same pathway.

We used coimmunoprecipitation and immunoblotting to test whether SLX5 and SLX8 encoded interacting proteins. A strain was constructed that expressed three epitope-tagged Slx proteins: Slx1-myc, Slx5-V5, and Slx8-HA. A cell lysate was incubated with anti-myc, anti-V5, or anti-HA antibody followed by immunoprecipitation and immunoblotting. As shown in Figure 9A, immunoprecipitation of Slx5-V5 coprecipitated Slx8-HA, whereasimmunoprecipitation of Slx1-myc did not. The reciprocal immunoprecipitation with anti-myc antibody confirmed the specificity of the interaction; neither Slx5-V5 nor Slx8-HA coprecipitated Slx1-myc. We conclude that Slx5 and Slx8 exist in a complex in the cell and suggest that, on the basis of their epistatic relationship, both proteins are required for activity of the complex.

Examination of Slx5 and Slx8 sequences revealed the presence of a RING finger type of zinc-binding domain in the C terminus of each protein (Figure 10A). This motif, Cys2-His-Cys2, is found in proteins with a variety of functions (e.g., BRCA1, PML1, Rbx1, and TRAF2) including some involved in DNA repair and recombination (e.g., Rad18 and RAG1; Borden and Freemont 1996). The precise function of the RING finger domain is unknown, but it has been suggested to mediate DNA binding or protein-protein interactions (Berg and Shi 1996). In the case of RAG1, the ring finger forms part of a specific dimerization domain (Rodgers et al. 1996). These domains may mediate the Slx5-8 interaction or an as yet unidentified interaction with DNA. A BLAST search identified potential homologs of Slx5 and Slx8 in S. pombe (Figure 10, B and C). Although the sequence similarity is low, the S. pombe Slx5-like sequence is comparable in size to the S. cerevisiae protein, and the similarity extends throughout the length of the protein (19% identical/43% similar) including a smaller RING finger motif (Figure 10B). The S. pombe Slx8-like sequence shows slightly better similarity to the S. cerevisiae protein (22% identical/42% similar), particularly in the RING finger domain (Figure 10C). Thus, Slx5 and Slx8 may be conserved in all eukaryotic species.

**Shared phenotypes of slx1Δ and slx4Δ mutants:** The slx1Δ and slx4Δ mutants differ from the other SLX mutants in that they displayed no increase in sensitivity to MMS or HU compared to wild type (Figure 3). In addition, these strains grow at wild-type rates and homzygous diploids sporulate at wild-type efficiencies (Table 3). We had previously used an slx4Δ strain to generate conditional alleles of SGS1 by screening for mutations that resulted in loss of viability in the slx4Δ background at 37°. One allele, sgs1-34, allowed the slx4Δ strain to grow at 25° but not at 37° and generated conditional sgs1 phenotypes in otherwise wild-type cells (V. Kalira and S. J. Brill, unpublished results). When this allele was tested in other slxΔ backgrounds we observed that mms4Δ sgs1-34 and slx3Δ sgs1-34 strains could grow at 37°, although at a reduced rate. In contrast, the slxΔ sgs1-34 strain was completely inviable at 37°, like the slx4Δ sgs1-34 strain (Table 3). We conclude that SLX1 and SLX4 define a third class of SLX genes.

To test whether SLX1 and SLX4 encoded interacting proteins, a strain expressing Slx1-myc and Slx4-HA was constructed and cell lysates were immunoprecipitated with anti-myc and anti-HA antibodies. As shown in Figure 9B, Slx4-HA protein could be coprecipitated with Slx1-myc using extracts derived from cells expressing both constructs. Slx4-HA migrated as a series of bands at ~120 kD, suggesting that it may be post-translationally modified (Figure 9B, left lanes). Similarly, the reciprocal experiment revealed that Slx1-myc could be immunoprecipitated with Slx4-HA when lysates were derived from cells containing both constructs (Figure 9B). We conclude, on the basis of coimmunoprecipitation and identical mutant phenotypes, that Slx1 and Slx4 exist
Mms4-HA:  +  +  +  
Slx3-V5: +  +  +  
IP: αHA  mock  αV5  

**Figure 7.** Mms4 and Slx3 proteins interact *in vivo*. Epitope-tagged versions of *MMS4* (HA) and *SLX3* (V5) were expressed under the *GAL* promoter in a wild-type yeast strain. Extracts were prepared and aliquots incubated with anti-HA, anti-V5, or no antibody as indicated. Protein A beads were incubated with all samples and washed under RIPA conditions. Precipitates were subjected to immunoblotting using anti-HA or anti-V5 antibody as probe.

![Blot Image](image)

**Figure 6.** Amino acid sequence comparison of Mms4 and Slx3 homologs. (A) The *S. cerevisiae* (AAF06816) and *N. crassa* (AL356173) potential Mms4 sequences were aligned with *S. cerevisiae* Rad10 (CAA86442) and human ERCC1 (NP001971) and identical or highly conserved residues are boxed. Presented is the C-terminal region of this alignment. (B) The following Slx3-related sequences were identified in a BLAST search using the *S. cerevisiae* Sbx3 protein (AAB64828) as query: *S. pombe* (CAB09772), *H. sapiens* (a, AA256727, H25808; b, A1174987), and *A. thaliana* (AL022198). A BLAST search with the highly conserved C-terminal domains of the potential Slx3 proteins as query identified similarity with the yeast Rad1 (AAB68165) and human XPF (AAB07689) proteins. All sequences were then aligned as in A.

**DISCUSSION**

The *SGS1* synthetic-lethal screen: The *in vivo* role of Sgs1 is not understood, although its enzymology as a DNA helicase (Lu et al. 1996; Bennett et al. 1998) and its mutant phenotypes of DNA damage sensitivity, poor sporulation, hyper-recombination, genome instability, and premature aging are well documented (Gangloff et al. 1994; Watt et al. 1995, 1996; Sinclair et al. 1997; Yamagata et al. 1998; Mullen et al. 2000). These phenotypes are consistent with the human diseases caused by mutations in the *recQ* family of genes—WRN, BLM, and RTS—and suggest that Sgs1 plays a role in DNA replication or repair. We expected that *sgs1* mutants would rely on known DNA repair or recombination pathways for survival and that the synthetic-lethal screen would identify genes in these major pathways. Surprisingly, our screen identified six genes of unknown function that were absolutely required in the absence of *SGS1*, and one mutant, *srs2*, that is synthetically sick in the absence of *SGS1*. Multiple isolates were obtained for all but one of the *SLX* mutants, showing that the mutant screen was close to saturation. Our data suggest that the six *SLX* genes encode three separate protein complexes. It is therefore possible that these protein complexes define all the factors required in the absence of *SGS1*.

Genetic analyses revealed that mutations in the six authentic *SLX* genes (*SLX1*, *MMS4*, *SLX3-5*, and *SLX8*) are lethal in the presence of a helicase-defective point mutation in *SGS1* or in the absence of *TOP3*. This result is consistent with accumulating evidence for genetic and physical interactions between Sgs1 and Top3. *SGS1* was together in a complex and suggest that both proteins are required for its activity.

The Slx4 protein sequence contains no known motifs while the Slx1 protein sequence contains a rare Cys3-His-Cys4 type zinc-binding domain at its C terminus (Figure 10A). A BLAST search identified potential homologs of Slx1 in humans and *C. elegans*. Comparison of these sequences identified large blocks of conserved sequence throughout the length of the proteins, including the zinc-binding domains (Figure 10D). Overall, the *C. elegans* and *Saccharomyces cerevisiae* proteins showed 27% identity and 54% similarity.

**Figure 8.** *SLX5* and *SLX8* mutants display a heterogeneous colony morphology. (Top) Wild-type (CHY128), *slx5* (JMY1221), *slx8* (JMY1222), and *slx5 slx8* (JMY1156) double mutants were streaked onto YPD plates and photographed after 2 (wt) or 4 (mutants) days at 30°. All mutant strains yielded a mixture of small and large colonies with uneven edges. (Bottom) A small and a large colony from the *slx5* strain was restreaked onto YPD, again yielding a similar mixture of colony sizes.
Further analysis revealed that the top3+ when the recombination rate is lowered to sgs1+ excessively high rates of recombination caused by the case/topoisomerase complex. At least two models have top3+ tants, this lethality was suppressed by sgs1+ top3+ mark the cysteine and histidine residues conserved in the zinc-binding motifs.

**SLX8**
c®c interactions with ony morphology, sporulation ef®ciency, and allele-spe-
other genetic phenotypes, including HU sensitivity, col-
epistatic in response to DNA damage. On the basis of 
form a unique phenotypic class and were shown to be 
SGS1+ C. elegans (D) Alignment of S. cerevisiae (AAC03214) and X. pombe (Z95620) potential Slx8 sequences. (D) Alignment of C. elegans (U80446), H. sapiens (H06103), and S. cerevisiae (CA85191) potential Slx1 sequences. Asterisks mark the cysteine and histidine residues conserved in the zinc-binding motifs.

Although six genes were identified in this study, the number of pathways required in the absence of SGS1 is likely to be far fewer. The MMS4 and SLX3 mutants form a unique phenotypic class and were shown to be epistatic in response to DNA damage. On the basis of other genetic phenotypes, including HU sensitivity, colony morphology, sporulation efficiency, and allele-specific interactions with sgs1-34, we found that SLX5 and SLX8 formed a second phenotypic class, while SLX1 and SLX4 comprised a third class. These conclusions were confirmed by coimmunoprecipitation studies of the encoded proteins: members of one phenotypic class precipitate with one another, but not with members of other classes. Thus, the three novel protein complexes we have identified are likely to define no more than three pathways required in the absence of SGS1/TOP3.

In addition to the six authentic SLX genes, three alleles of SRS2/HPR5 were isolated in the screen. Our finding that the srs2Δ sgs1Δ double mutant is synthet-
ically sick is consistent with the results of Gangloff et al. (2000) but differs from those of Lee et al. (1999), who concluded that at least one of these genes is essential for viability and for DNA replication fork movement. Although we cannot rule out the presence of a suppressor in our background, we find it unlikely that a suppressor could easily arise to function as a replicative DNA helicase. It is likely that additional background mutations in the original EMS-mutagenized strain resulted in the requirement for SGS1 in these srs2 sgs1Δ isolates. Further analysis revealed that the top3Δ srs2Δ double mutant was inviable, but unlike other top3Δ sgs1Δ mutants, this lethality was suppressed by sgs1Δ (Figure 2C). We suggest that the top3Δ srs2Δ strain is inviable due to excessively high rates of recombination caused by the additive effects of two hyper-recombination mutations. When the recombination rate is lowered to sgs1 levels by adding the sgs1 mutation (Gangloff et al. 1994), viability is restored to the srs2Δ top3Δ sgs1Δ triple mutant. This model is consistent with the recent finding that reducing homologous recombination suppresses the slow growth of the srs2Δ sgs1Δ double mutant (Gangloff et al. 2000).

**Functions of SLX proteins:** The functions of the Slx proteins are unknown, but on the basis of their involvement in DNA damage response and sporulation, they are likely to be related to those of the Sgs1/Top3 heli-
case/topoisomerase complex. At least two models have been proposed to explain the role of Sgs1/Top3. One model proposes that these enzymes act at the termina-

**Figure 9.—** Slx5/8 and Slx1/4 proteins interact in vivo. (A) Epitope-tagged versions of SLX1 (myc), SLX5 (V5), and SLX8 (HA) were expressed under the GAL promoter in a wild-type yeast strain. Extracts were prepared and aliquots were incubated with anti-myc, anti-V5, or anti-HA as indicated. Protein A beads were incubated with each sample and washed under RIPA conditions. Precipitates were subjected to immunoblotting using anti-myc or anti-HA antibody as probe. Non-specific antibody bands are indicated. (B) Epitope-tagged versions of SLX1 (myc) and SLX4 (HA) were expressed and immunoprecipitated with the indicated antibodies, as described above. Precipitates were subjected to immunoblotting using anti-HA or anti-myc antibody as probe.

**Figure 10.—** Amino acid sequence analysis of Slx1, Slx5, and Slx8. (A) Consensus sequences for the zinc-binding motifs found in Slx5, Slx8, and Slx1. C, cysteine; H, histidine; X, any amino acid. (B) Alignment of S. cerevisiae (CA98570) and S. pombe (AL03570) potential Slx5 sequences. (C) Alignment of S. cerevisiae (AAC03214) and X. pombe (Z95620) potential Slx8 sequences. (D) Alignment of C. elegans (U80446), H. sapiens (H06103), and S. cerevisiae (CA85191) potential Slx1 sequences. Asterisks mark the cysteine and histidine residues conserved in the zinc-binding motifs.
Genes Required in the Absence of SGS1

Slx5: C-X2-C-X12.58-C-X1-H-X2-C-X2-C-X11.34-C-X2.4-C
Slx8: C-X2-C-X11-C-X1-H-X2-C-X2-C-X13.16-C-X2-C
Slx1: C-X2-C-X12.19-C-X4-C-X4-H-X2-C-X19.24-C-X2-C
tion of DNA replication when daughter chromosomes become entangled as replication forks converge. It is thought that there are two pathways of resolution: DNA-topoisomerase II-mediated decatenation of intertwined daughter chromosomes following completion of DNA synthesis or a topoisomerase-III-mediated decatenation of template strands prior to completion of DNA synthesis (Wang 1991; Rothstein and Gangloff 1995). The Slx proteins might participate in synthesis of the final DNA strands at converging replication forks, perhaps working with a DNA helicase. In this way daughter chromosomes would become fully duplex and, subsequently, substrates for decatenation by Top2. In the absence of complete DNA synthesis, the cell would become dependent on the Sgs1/Top3 pathway for decatenation.

A second model for Sgs1/Top3 is that it acts during DNA replication to repair stalled replication forks. It is known that some members of the recF recombination pathway are required to restart stalled replication forks in E. coli (Courcelle et al. 1997) and that recQ and recF are needed for the normal degradation of nascent lagging strands at stalled replication forks (Courcelle and Hanawalt 1999). The eukaryotic recQ homologs, like SGS1, may perform a similar function (Ghakraverty and Hickson 1999). It has also been suggested that the BLM helicase might act to restart stalled replication forks by branch-migrating Holliday junctions that form at stalled forks (Karow et al. 2000). Such a role for Sgs1/Top3 and the Slx proteins could explain the DNA damage and HU sensitivity of these mutants. Based on this, our preferred model, the Slx proteins might act to modify the stalled structure when Sgs1/Top3 activity is absent. The failure to modify these structures during premeiotic S phase would be expected to reduce or eliminate sporulation, thereby explaining the sporulation defects of certain SLX mutants.

The enzymatic activities of the Slx proteins cannot be deduced from their amino acid sequences and it remains possible that they require additional proteins for activity. However, we feel that the pairing of mutant phenotypes is not a coincidence and that the Slx proteins are likely to function as heterodimers for the following reasons. First, the mutant screen was nearly saturated and if other equally important subunits existed, they likely would have been identified. Second, preliminary data from our lab indicate that Mms4/Slx3 and Slx1/4 pairs form soluble heterodimeric complexes when expressed as recombinant proteins in E. coli (our unpublished results). While the three pairs could represent three separate pathways, we suggest for simplicity that the three complexes act in one pathway and generate different phenotypes on the basis of their order of function. These protein dimers might interact with a substrate containing a two-fold symmetry, such as a replication fork or recombination intermediate. Alternatively, the heterodimeric structures might reflect coordinate regulation in which each complex contains both a catalytic and a regulatory subunit.

The Mms4 and Slx3 proteins present the best opportunity for predicting function from amino acid sequence. These two proteins physically interact and share weak similarity to the Rad1/Rad10 endonuclease of yeast. On the basis of this information we suggest that Mms4 is not a transcription factor as originally proposed (Xiao et al. 1998) but that Mms4/Slx3 is a heterodimeric endonuclease. Mms4/Slx3 is unlikely to function in nucleotide excision repair given the mutants’ weak UV-sensitive phenotype. However, this nuclease may function in response to DNA damage, similarly to the role of Sgs1/Top3. One possibility is that Mms4/Slx3 recognizes a specific structure during recombinational repair and nicks one or more strands to allow proper chromosomal segregation. As stated above, the failure to resolve recombination intermediates would lead to exactly the defects in meiosis and sporulation that are observed in these mutants. Biochemical experiments will be needed to test this prediction while genetic experiments will be needed to search for unresolved recombination intermediates in SLX mutants.

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