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 Slx3 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 Slx5 and Slx8 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 Slx1 and Slx4 proteins are similarly associated in cell extracts. We propose that the *MMS4/SLX3*, *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*.

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.* 1990) 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 increased rates of sister chromatid exchange, a mutator phenotype, and slowed DNA replication (Chaganti *et al.* 1974; Hand and German 1975; Vijayalaxmi *et al.* 1983). Werner's syndrome cell lines (*WRN*) display variegated translocation mosaicism, slowed DNA replication, and sensitivity to the DNA-damaging agent 4-NQO (Fujiwara *et al.* 1977; Salk *et al.* 1981; Gebhart *et al.* 1988). Rothmund-Thomson syndrome fibroblasts (*RecQL4*) show elevated rates of chromosomal breaks and rearrangements (Miozzo *et al.* 1998).

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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 from *E. coli*. Both proteins have weak superhelical relaxing activity *in vitro* and a strict requirement for substrates containing single-stranded DNA (ssDNA) for strand-passing activity (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 from *E. coli* or yeast to catenate double-stranded DNA circles (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 (*s*ynthetic *l*ethal 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*.

MATERIALS AND METHODS

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 *Bam*HI/*Sal*I *ADE3* fragment and a *Xho*I/*Sac*I *SGS1* PCR product into *Bam*HI/*Sac*I-cut pRS416 (Sikorski and Hieter 1989). pJM501 was constructed by ligating the *Xho*I/*Sac*I *SGS1* PCR product into *Xho*I/*Sac*I-cut pRS413. Plasmids pJM555 and pJM6710 were described previously (Mullen *et al.* 2000), as were pJL31 and pJL37 (Lu *et al.* 1996).

Isolation of synthetic lethal mutants: JMY329α and JMY332**a** 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 $\sim 2 \times 10^8$ cells/ml, washed, and concentrated to $\sim 8 \times 10^9$ cells/ml in 100 mM $NaPO_4$ 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°. 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 JMY329 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 (FOA^S) strains were transformed with pJM501 (*SGS1-HIS3*) and restreaked onto FOA. Strains that became FOA resistant (FOA^R) 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**

Strain	Genotype	Reference or source
W303-1a	*MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Thomas and Rothstein (1989)
RS190	W303-1a *top1-8::LEU2*	Brill and Sternglanz (1988)
CHY128	*MAT*α *ade2-1 ade3::hisG ura3-1 his3-11,15 leu2-3,112 lys2 can1-100*	Chris Hardy
CHY125	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	Mullen *et al.* (2000)
HKY715	*MAT***a** *ura3-1 leu2-3,112 his3-11,15 trp1::hisG srs2::TRP1 can1::hisG*	Hannah Klein
JMY329	*MAT*α *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 lys2 can1-100 sgs1-3::TRP1* + pJM500 (*SGS1/URA3/ADE3*)	This study
JMY331	W303-1a *sgs1-3::TRP1 top1-8::LEU2*	This study
JMY332	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-3::TRP1* + pJM500 (*SGS1/URA3/ADE3*)	This study
JMY360	W303-1a *slx1-10::TRP1*	This study
JMY361	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 slx1-10::TRP1*	This study
JMY373	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 top3-2::HIS3*	This study
JMY375	W303-1a *slx2-10::KAN*	This study
JMY380	W303-1a *slx3-10::KAN*	This study
JMY422	*MAT***a** *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*)	This study
JMY442	W303-1a *slx2-10::KAN top1-8::LEU2*	This study
JMY446	W303-1a *slx3-10::KAN top1-8::LEU2*	This study
NJY510	*MAT*α *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 lys2 can1-100 sgs1-11::KAN, loxP*	This study
NJY517	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11, trp1-1 leu2-3,112 can1-100 slx4-11::KAN*	This study
NJY531	CHY125 *sgs1-11::loxP*	Mullen *et al.* (2000)
NJY566	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 top3-11::loxP slx4-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY593	*MAT*α *ade2-1 ade3::hisG ura3-1 his3-20::loxP trp1-11::loxP leu2-3,112 can1-100 slx5-10::TRP1*	This study
NJY608	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 slx4-11::loxP sgs1-11::loxP top3-11::loxP* + pJM6401 (*SLX4/URA3/ADE3*)	This study
NJY612	*MAT*α *ade2-1 ade3-hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 slx2-10::KAN top3-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY613	*MAT*α *ade2-1 ade3-hisG ura3-1 his3-11,15 trp1-1 lys2 can1-100 slx3-10::KAN top3-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY625	*MAT*α *ade2-1 ade3::hisG ura3-1 his3 trp1 lys2 can1-100 slx5-10::TRP1 top3-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
SIY778	W303-1a *slx8-11::KAN, loxP*	This study
NJY1061	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 srs2::TRP1 top3-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY1109	*MAT*α *ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 srs2::TRP1 sgs1-11::KAN, loxP top3-11::loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY1113	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 top3-2::HIS3 slx8-11::KAN, loxP* + pJM555 (*TOP3/URA3/ADE3*)	This study
NJY1116	*MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sgs1-3::TRP1 top3-2::HIS3 slx2-10::KAN* + pNJ6211 (*SLX2/URA3*)	This study
NJY1118	*MAT***a** *ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 sgs1-3::TRP1 top3-2::HIS3 slx3-10::KAN* + pJM6308 (*SLX3/URA3*)	This study
JMY1156	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3 trp1 leu2-3,112 can1-100 slx5-10::TRP1 slx8-11::KAN, loxP*	This study
JMY1221	*MAT***a** *ade2-1 ade3::hisG ura3-1 his3 trp1 leu2-3,112 can1-100 slx5-10::TRP1*	This study
JMY1222	*MAT*α *ade2-1 ade3::hisG ura3-1 his3 trp1 lys2 can1-100 slx8-11::KAN, loxP*	This study

locus as follows. The wild-type *SLX* ORF in an *sgs1*Δ mutant was marked (but not disrupted) with *HIS3* by integrative transformation, crossed to the respective mutant (*sgs1*Δ *slx his3* + pJM500), and the diploid was subjected to tetrad analysis. Linkage analysis results were as follows: *SLX1*: (51 spores) 35 FOA^R-His^+, 16 FOA^S-15 His^-, 1 FOA^S-His^+. *SLX2*: (37 spores) 21 FOA^R-His^+, 10 FOA^S-His^-, 6 FOA^R-His^-. *SLX3*: (46 spores) 26 FOA^R-His^+, 20 FOA^S-His^-. *SLX4*: (26 spores) 17 FOA^R-His^+, 9 FOA^S-His^-. *SLX5*: (34 spores) 18 FOA^R-His^+, 16 FOA^S-His^-. *SLX8*: (78 spores) 44 FOA^R-His^+, 34 FOA^S-His^-. For each *SLX* gene, the synthetic lethal phenotype was confirmed by generating an *slx* null allele, crossing it to an *sgs1*Δ mutant, and analyzing tetrads. All *slx sgs1* double-mutant spores failed to form colonies.

***SLX* deletions:** Two alleles of *SLX1* were made: *slx1-10::TRP1* and *slx1-11::HIS3*. To create *slx1-11::HIS3*, a *Bgl*II site was intro-

duced after amino acid 30 of *SLX1* by PCR and a *HIS3* fragment was ligated into the *Bgl*II/*Xho*I-cut *SLX1* gene (*Xho*I cuts at amino acid 274 out of 304). To create *slx1-10::TRP1*, a *Bam*HI/*Xho*I *TRP1* fragment was cloned into the same *Bgl*II/*Xho*I-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 *SLX1* 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-SLX1-13MYC*). The *MMS4* gene and a fragment encoding a C-terminal triple-hemagglutinin (HA) tag was placed under the *GAL/Phosphoglucokinase* (*PGK*) promoter of plasmid pHG2 (2μ, *LEU2-d, GAL-PGK-6His*) 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 *SLX4* gene was subcloned into plasmid pNJ6209 to create pNJ6409 (2μ, *LEU2-d, GAL-PGK-6His-SLX4-3HA*). 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° to ~1×10^7 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°. 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 *sgs1*Δ *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 *slx* 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 *slx*Δ deletions were created and all were found to be viable in the wt *SGS1* background (Table 2). Six of the seven *slx*Δ null mutations were inviable in combination with *sgs1*Δ (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 two top panels of Figure 1. For simplicity, only an *mms4*Δ *sgs1*Δ strain is presented, but all of the double mutants behave identically in this regard (Figure 1 and data not shown). An *mms4*Δ *sgs1*Δ 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 allow 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 *slx*Δ mutants require the Sgs1 protein and, specifically, the helicase activity of Sgs1 to survive (Table 2).

We examined the *mms4*Δ *sgs1*Δ 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 *sgs1*Δ 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)

TABLE 2

***SGS1* synthetic-lethal mutants**

Complementation group	No. of alleles	Mutant gene	Complementation with *sgs1* alleles[a]					
			SGS1	*sgs1*Δ	*sgs1-hd*	*sgs1-ΔN158*	*sgs1-ΔC200*	*sgs1-ΔC300*
SLX1	8	YBR228W	+	−	−	−	+	−
SLX2	7	*MMS4*	+	−	−	−	+	−
SLX3	6	YDR386W	+	−	−	−	+	−
SLX4	6	YLR135W	+	−	−	−	+	−
SLX5	3	YDL013W	+	−	−	−	+	−
SLX6	3	*SRS2/HPR5*	+	+[b]	+	ND	ND	ND
SLX8	1	YER116C	+	−	−	ND	ND	ND

[a] *slx*Δ *sgs1*Δ complementation by the indicated *SGS1* allele: +, viable; −, lethal; ND, not done.
[b] *srs2*Δ *sgs1*Δ 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 *slx*Δ *sgs1*Δ 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 *slx*Δ 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, *slx*Δ 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), *dna2-1*^{ts} (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 *mms4*Δ or *slx3*Δ. 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

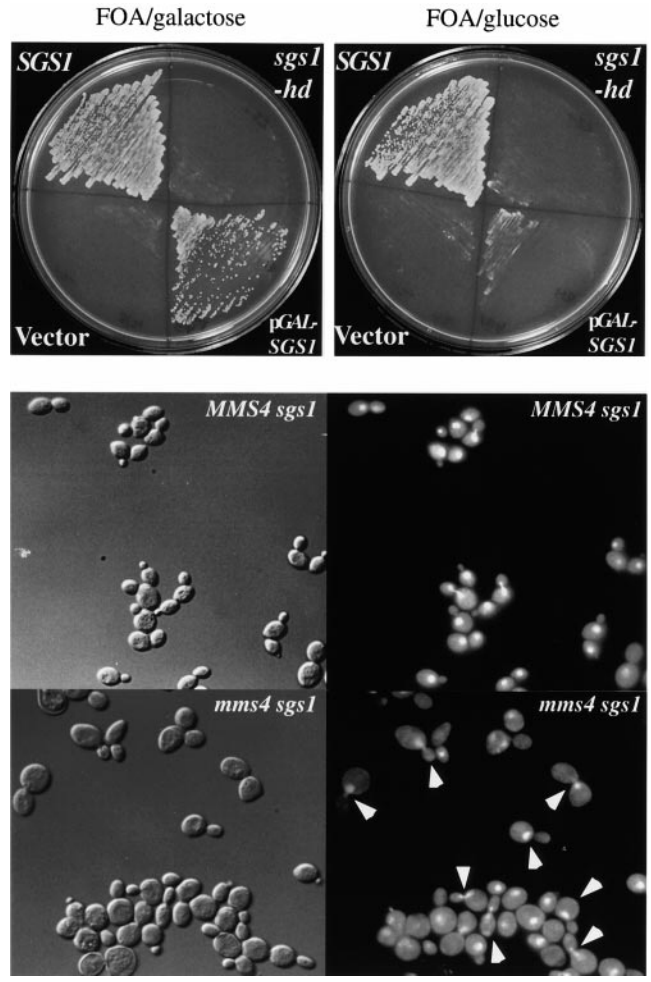


Figure 1.—*slx* mutants require *SGS1* function for viability. (Top) JMY422 (*mms4 sgs1* + pJM500) transformed with pJL31 (*SGS1-LEU2*), pJL37 (*sgs1-hd-LEU2*), pJM6710 (*GAL-SGS1-LEU2*), and pRS415, streaked onto indicated plates containing FOA (to select against pJM500), and incubated at 30° for 4 days. (Middle) JMY332 (*sgs1*) cells; (bottom) JMY422 cells, both carrying pJM6710, after growth in glucose for 2 hr. DIC micrographs are shown on the left and DAPI staining on the right. Arrowheads indicate cells with DNA trapped in the neck between mother and daughter.

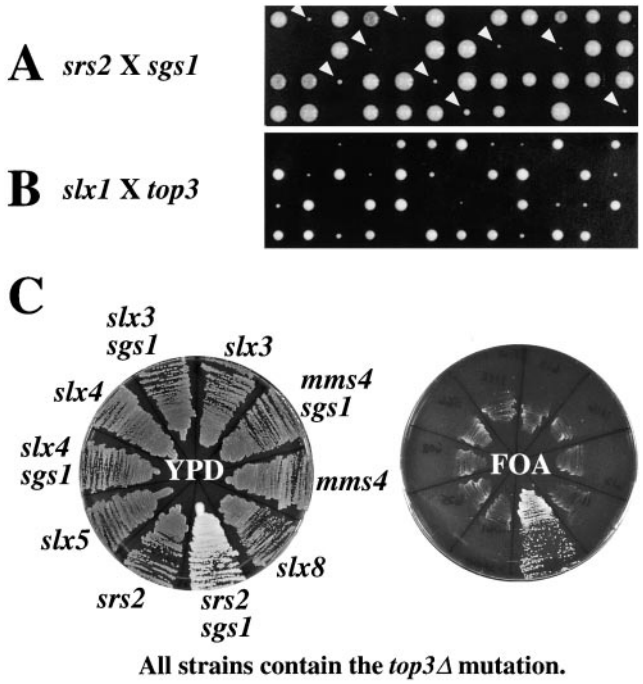


FIGURE 2.—Genetic interactions between *SLX* and *SGS1/TOP3* mutations. (A) Tetrads from a cross between strains HKY715 (*srs2*) and NJY510 (*sgs1*) were dissected and incubated for 4 days at 30°. Subsequent analysis revealed that small colonies (indicated by arrowheads) are *srs2 sgs1* double mutants. (B) Tetrads from a cross between JMY361 (*slx1*) and JMY373 (*top3*) were dissected and incubated for 3 days at 30°. Subsequent analysis revealed that small colonies are *top3* mutants and all dead spores are *slx1 top3* double mutants. (C) Yeast *top3* strains with the indicated additional mutations were streaked onto YPD and FOA plates and incubated for 3 days at 30°. Double-mutant strains contain a *TOP3/URA3* plasmid (pJM555), while triple-mutant strains contain the respective *SLX/URA3* plasmid.

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 *srs2*Δ *sgs1*Δ 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 *slx*Δ mutant was crossed to a *top3*Δ 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 *top3*Δ and each of the *slx*Δ 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 *sgs1*Δ mutation to *mms4*Δ *top3*Δ, *slx3*Δ *top3*Δ, or *slx4*Δ *top3*Δ double mutants did not suppress lethality (Figure 2C). In contrast, adding the *sgs1*Δ mutation to the *top3*Δ *srs2*Δ 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 *slx*Δ strains had any phenotypes in common with *sgs1*Δ strains. Wild-type yeast cells are able to grow in the presence of methyl methansulfonate (MMS) and hydroxyurea (HU), whereas *sgs1* strains are hypersensitive to these drugs and grow poorly in their presence (YAMAGATA *et al.* 1998; MULLEN *et al.* 2000). To test the *slx*Δ 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°, the *mms4*Δ and *slx3*Δ strains grew very poorly on the MMS plate (Figure 3, left). On the basis of this assay, the *mms4*Δ and *slx3*Δ strains are about as sensitive to MMS as is the *sgs1*Δ strain. This phenotype is not true of all *slx*Δ 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 *slx*Δ strains were similarly tested for HU sensitivity. In the presence of HU the growth of *slx5*Δ and *slx8*Δ strains was strongly inhibited, as was the growth of the *sgs1*Δ strain (Figure 3, middle). In contrast, the growth of *mms4*Δ and *slx3*Δ mutants was only weakly inhibited, while the growth of *slx1*Δ and *slx4*Δ 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 *mms4*Δ and *slx3*Δ 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° for 2 days, and colonies were counted. Two known UV-sensitive strains, *rad3* and *rad9*, were included as controls. The *mms4*Δ and *slx3*Δ strains showed identical profiles of weak UV sensitivity. At the highest UV dose, the *mms4*Δ and *slx3*Δ strains were ~40 times more sensitive than the wild-type parent and ~20 times less

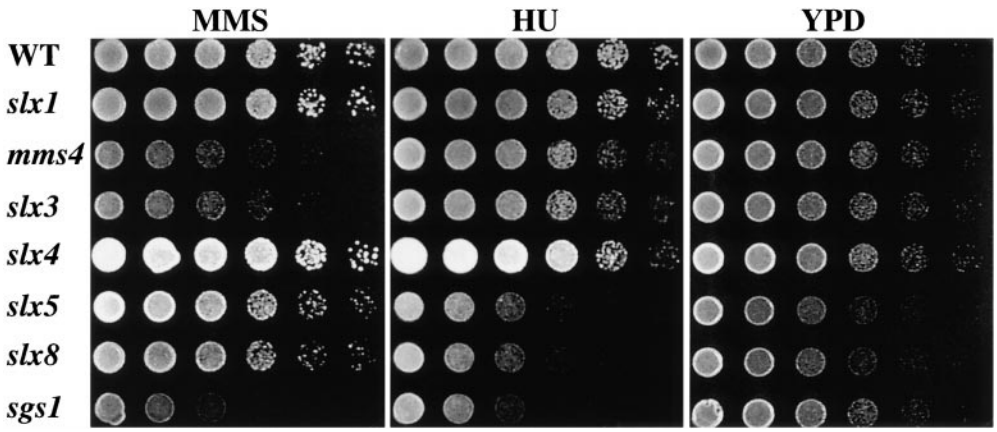


FIGURE 3.—Pairs of *SLX* mutants display hypersensitivity to MMS and HU. The indicated strains were taken from a freshly growing YPD plate and diluted in water to $OD_{600} = 5.0$. Serial 1:5 dilutions were made in a microtiter plate and 5-μl volumes were replica plated onto YPD plates with or without 0.012% MMS or 100 mM HU as indicated. Plates were photographed after 1 day (YPD), 2 days (MMS), or 4 days (HU) at 30°. Like *sgs1* mutants, *mms4* and *slx3* mutants display hypersensitivity to MMS, while *slx5* and *slx8* mutants display hypersensitivity to HU.

sensitive than the *rad9* mutant (Figure 4A). The *mms4*Δ *slx3*Δ 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 *mms4*Δ and *slx3*Δ mutants, assays in liquid culture were performed. After 1 hr in 0.3% MMS, both the *mms4*Δ and *slx3*Δ mutants were ~50 times more sensitive than their isogenic parent (Figure 4B). Once again, the *mms4*Δ *slx3*Δ 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 *slx*Δ mutants were crossed to a *top1*Δ *top2-1(ts)* strain and the resulting tetrads were analyzed. Although the *slx1*Δ mutation was unaffected by the addition of either *top1*Δ or *top2-1(ts)* (Table 3), we found that both *mms4*Δ and *slx3*Δ interacted genetically with mutations in *TOP1* and *TOP2*. The *mms4*Δ *top1*Δ and *slx3*Δ *top1*Δ strains grew as slowly as the *sgs1*Δ *top1*Δ double mutant and more slowly than either single mutant (Figure 5). In addition, both *mms4*Δ *top2-1* and *slx3*Δ *top2-1* strains grew more slowly than single mutants at the semipermissive temperature of 30° (data not shown).

Diploid strains homozygous for each of the *slx*Δ mutations were constructed and tested for their ability to sporulate. Diploids that were homozygous mutant for *slx1*Δ and *slx4*Δ were able to sporulate like wild type, but *mms4*Δ and *slx3*Δ homozygous diploids were completely defective and formed no complete asci (Table 3). Interestingly, *mms4*Δ and *slx3*Δ diploids gave rise to rare two-spored asci 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 *mms4*Δ and *slx3*Δ diploids are defective in meiosis and not spore formation. Additional tests on the *mms4*Δ and *slx3*Δ strains showed no significant effect on telomere length, heat shock sensitivity, growth at 37° and 15°, growth on glycerol, 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;

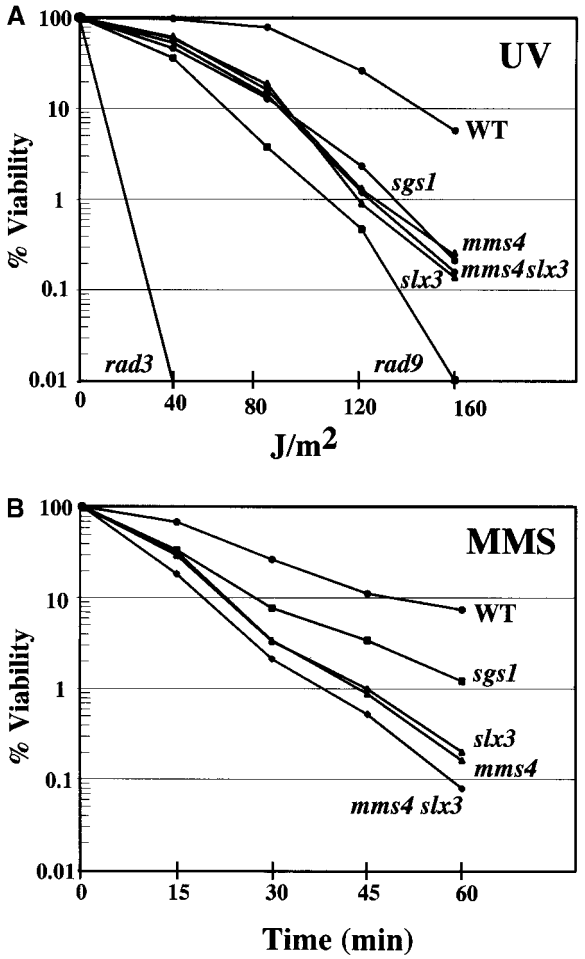


FIGURE 4.—Mutations in *MMS4* and *SLX3* are epistatic in response to DNA damage. (A) Exponentially growing cells were diluted appropriately in water, plated, exposed to increasing amounts of UV radiation, and incubated at 30° for 3 days. Viable colonies were counted and expressed as percentage viability 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.

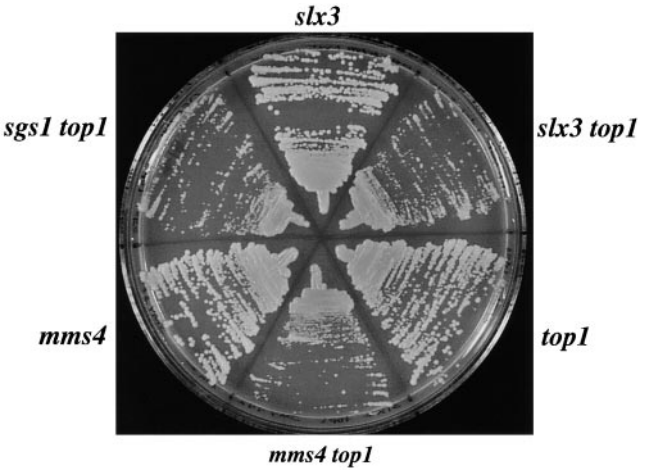


FIGURE 5.—*MMS4* and *SLX3* interact with *TOP1*. The indicated single- and double-mutant strains were streaked onto YPD and grown at 30° for 2 days. As previously shown, the *sgs1 top1* double mutant (JMY331) grows more slowly than either the *top1* (RS190) or *sgs1* (not shown) single mutants. The *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.

BAILIS and ROTHSTEIN 1990). The C termini of both Rad1 and Rad10 are known to be evolutionarily conserved and to be required for their dimerization (BARDWELL *et al.* 1993). Interestingly, the region of Rad1 with greatest similarity to Slx3 (aa 823–1047) corresponds closely to the Rad10-binding region (aa 809–997, BARDWELL *et al.* 1993). Given that Mms4 and Slx3 are epistatic, we looked for any sequence similarities between Mms4 and the Rad10 protein (210 aa). An alignment between Mms4 and Rad10 found a weak but identifiable sequence similarity between the C termini of these proteins (Figure 6A). Intriguingly, the region of Rad10 with greatest similarity to Mms4 (aa 93–210) corresponds closely to the Rad1-binding region of Rad10 (aa 90–210, BARDWELL *et al.* 1993). Although the level of conservation is low, the region of similarity is extended when Mms4 is compared to the human Rad10 homolog ERCC1 (Figure 6A).

This sequence analysis suggests that Mms4 and Slx3 exist as a complex *in vivo*. To test this, epitope-tagged versions of the *MMS4* and *SLX3* genes were coexpressed in yeast and subjected to immunoprecipitation (IP) and immunoblotting. Figure 7 demonstrates that immunoprecipitation of Mms4-HA results in the coprecipitation of Slx3-V5. The reciprocal experiment confirms that Mms4-HA coprecipitates with Slx3-V5 and that neither protein is detected when antibody is omitted from the IP. We conclude that Mms4 and Slx3 are present in a complex *in vivo* and suggest that the simplest explanation for their epistatic interaction is that both proteins are required for the activity of the complex.

Shared phenotypes of *slx5*Δ and *slx8*Δ mutants: Mutations in *SLX5* and *SLX8* generate very similar levels of HU sensitivity (Figure 3), suggesting that these two genes are involved in the same process. This idea was further supported by the growth phenotype of these mutants. The growth rate and plating efficiency of *slx5* and *slx8* strains are reduced relative to wild type (data not shown), and both strains produce a unique colony morphology when streaked on solid media. Whereas wild-type cells give rise to colonies of uniform size and round shape, *slx5* and *slx8* mutants form a mixture of large and small colonies with rough edges (Figure 8, top). Furthermore, for both mutants the colony size did not breed true; restreaking of either small or large colonies gave rise to a similar mixture of colony sizes (Figure 8, bottom and data not shown). Finally, the mixed colony size phenotype was not exacerbated in the *slx5*Δ *slx8*Δ double mutant, again suggesting that

TABLE 3

Summary of shared *SLX* mutant phenotypes

Mutant gene	Growth[a]	MMS[b]	HU[b]	*top1* synthetic sickness	Sporulation (% wt)	*sgs1-34* synthetic lethality[c]
MMS4	+	−	±	Yes	0	+
SLX3	+	−	±	Yes	0	+
SLX5	S	+	−	ND	30	−
SLX8	S	+	−	ND	30	ND
SLX1	+	+	+	No	100	−
SLX4	+	+	+	ND	100	−

[a] +, wt growth; S, slow growth/heterogeneous colony morphology.
[b] +, insensitive; ±, partially sensitive; −, very sensitive.
[c] +, viable at 37°; −, inviable at 37°; ND, not done.

these genes function in the same genetic pathway. Diploids homozygous for either *slx5*Δ or *slx8*Δ were tested for sporulation efficiency. In contrast to *mms4*Δ and *slx3*Δ 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.

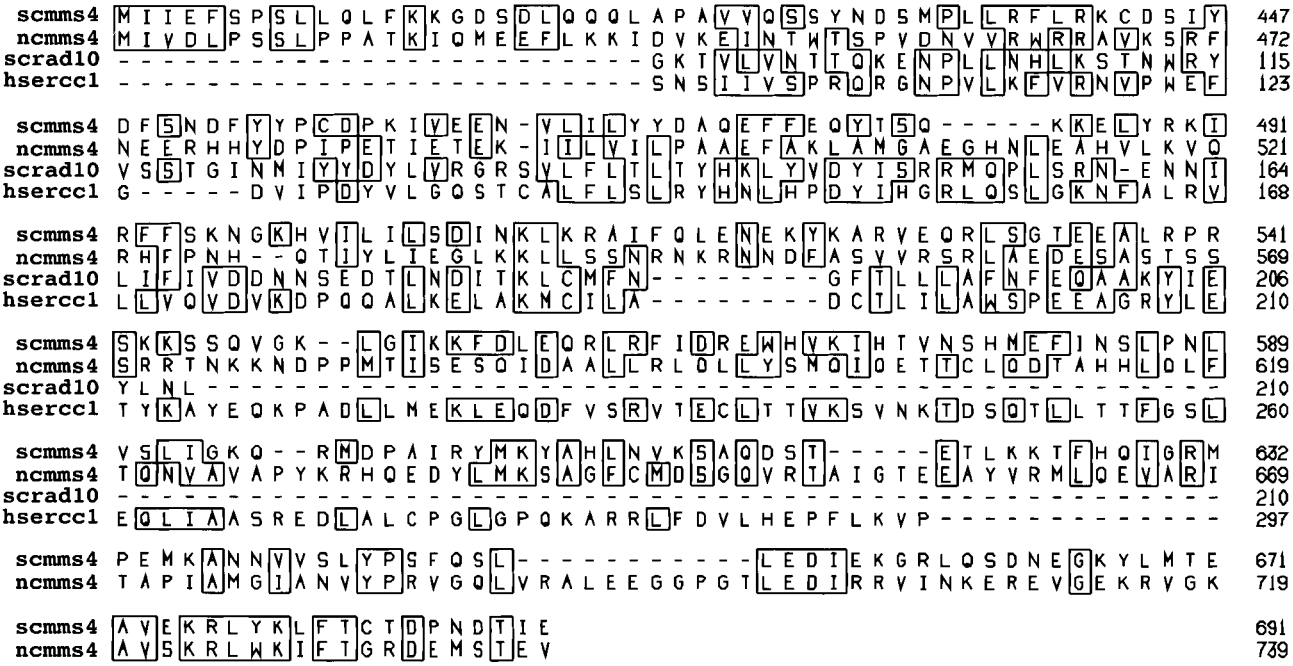
We used coimmunoprecipitation and immunoblotting to test whether *SLX5* and *SLX8* encoded interacting proteins. A strain was constructed that expressed three epitope-tagged Slx proteins: Slx1-myc, Slx5-V5, and Slx8-HA. A cell lysate was incubated with anti-myc, anti-V5, or anti-HA antibody followed by immunoprecipitation and immunoblotting. As shown in Figure 9A, immunoprecipitation of Slx5-V5 coprecipitated Slx8-HA, whereas immunoprecipitation 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, Cys_3-His-Cys_4, 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 *slx1*Δ and *slx4*Δ mutants: The *slx1*Δ and *slx4*Δ 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 homozygous diploids sporulate at wild-type efficiencies (Table 3). We had previously used an *slx4*Δ strain to generate conditional alleles of *SGS1* by screening for mutations that resulted in loss of viability in the *slx4*Δ background at 37°. One allele, *sgs1-34*, allowed the *slx4*Δ strain to grow at 25° but not at 37° and generated conditional *sgs1* phenotypes in otherwise wild-type cells (V. Kaliraman and S. J. Brill, unpublished results). When this allele was tested in other *slx*Δ backgrounds we observed that *mms4*Δ *sgs1-34* and *slx3*Δ *sgs1-34* strains could grow at 37°, although at a reduced rate. In contrast, the *slx1*Δ *sgs1-34* strain was completely inviable at 37°, like the *slx4*Δ *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

A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scrad10
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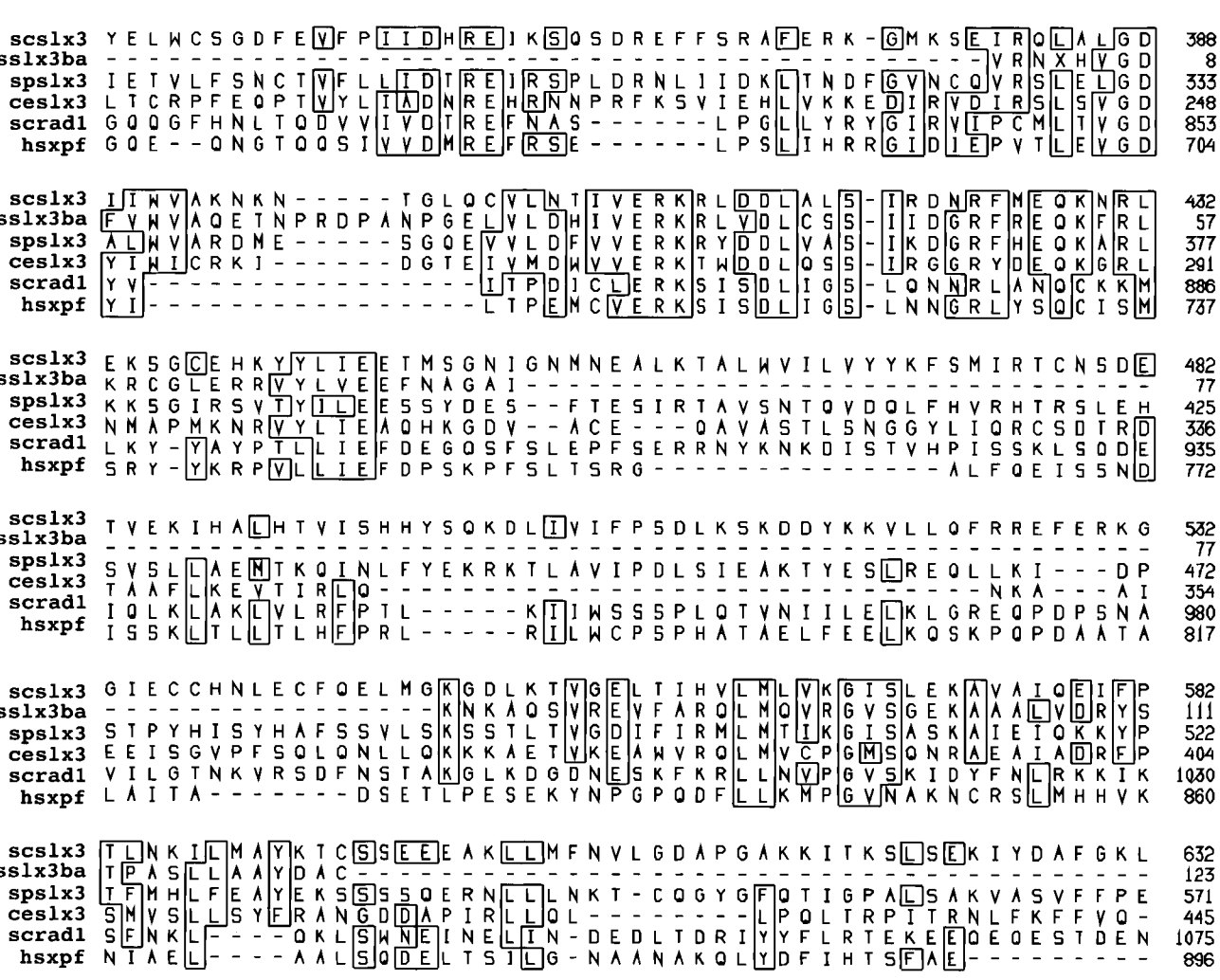
B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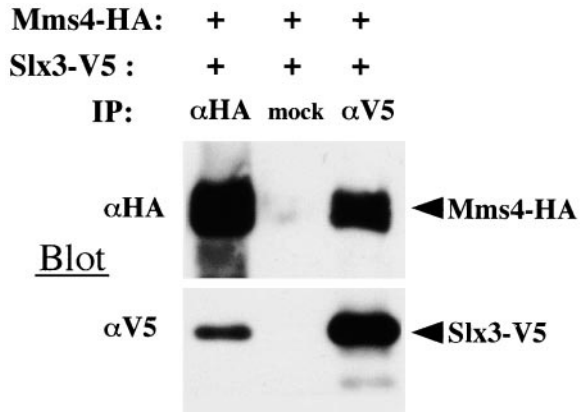


FIGURE 7.—Mms4 and Slx3 proteins interact *in vivo*. Epitope-tagged versions of *MMS4* (HA) and *SLX3* (V5) were expressed under the *GAL* promoter in a wild-type yeast strain. Extracts were prepared and aliquots incubated with anti-HA, anti-V5, or no antibody as indicated. Protein A beads were incubated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

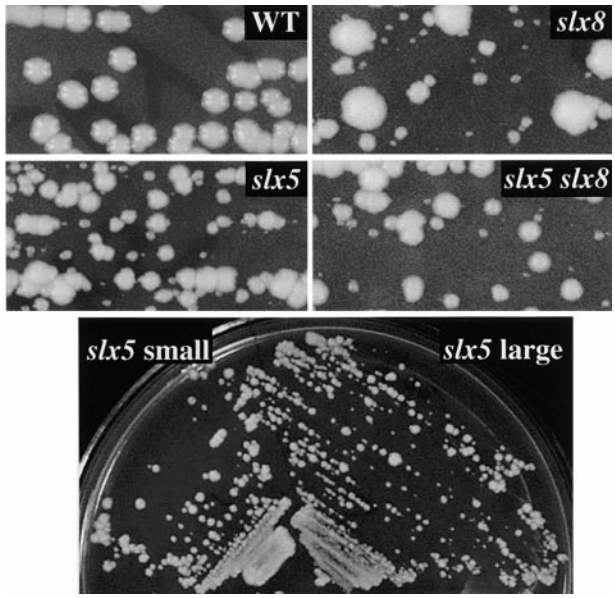


FIGURE 8.—*SLX5* and *SLX8* mutants display a heterogeneous colony morphology. (Top) Wild-type (CHY128), *slx5* (JMY1221), *slx8* (JMY1222), and *slx5 slx8* (JMY1156) double mutants were streaked onto YPD plates and photographed after 2 (wt) or 4 (mutants) days at 30°. All mutant strains yielded a mixture 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.

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 Cys_4-His-Cys_3 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.

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

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 (CAA86642) 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* Slx3 protein (AAB64828) as query: *S. pombe* (CAB09772), *C. elegans* (AAB37627), *H. sapiens* (a, AA256727, H25803; b, AI174987), 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.

originally identified on the basis of its genetic and two-hybrid interactions with *TOP3* (Gangloff *et al.* 1994). Recently, an *in vitro* interaction between Sgs1 and Top3 was reported (Bennett *et al.* 2000) and confirmed by immunoprecipitation and cofractionation of cell extracts in our lab (W. Fricke and S. J. Brill, unpublished results). Similarly, it has been shown that the Blm protein co-localizes with human topoisomerase IIIα *in vivo* and binds to it *in vitro* (Johnson *et al.* 2000; Wu *et al.* 2000). The fact that *SLX* mutations interact with mutations in both known components of this helicase/topoisomerase complex is consistent with the idea that the *SLX* genes function in a pathway parallel to that of *SGS1/TOP3*.

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 *srs2*Δ *sgs1*Δ double mutant is synthetically 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 sgs1*Δ isolates. Further analysis revealed that the *top3*Δ *srs2*Δ double mutant was inviable, but unlike other *top3*Δ *slx*Δ mutants, this lethality was suppressed by *sgs1*Δ (Figure 2C). We suggest that the *top3*Δ *srs2*Δ 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 *srs2*Δ *top3*Δ *sgs1*Δ triple mutant. This model is consistent with the recent finding that reducing homologous recombination suppresses the slow growth of the *srs2*Δ *sgs1*Δ double mutant (Gangloff *et al.* 2000).

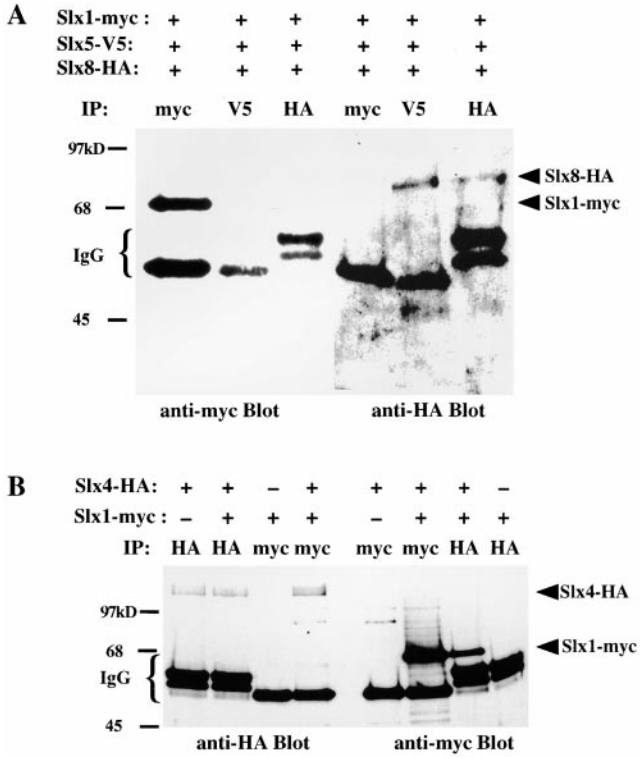


Figure 9.—Slx5/8 and Slx1/4 proteins interact *in vivo*. (A) Epitope-tagged versions of *SLX1* (myc), *SLX5* (V5), and *SLX8* (HA) were expressed under the *GAL* promoter in a wild-type yeast strain. Extracts were prepared and aliquots were incubated with anti-myc, anti-V5, or anti-HA as indicated. Protein 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. Nonspecific 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.

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 helicase/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 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* (CAA98570) and *S. pombe* (AL035075) potential Slx5 sequences. (C) Alignment of *S. cerevisiae* (AAC03214) and *S. pombe* (Z95620) potential Slx8 sequences. (D) Alignment of *C. elegans* (U80446), *H. sapiens* (H06103), and *S. cerevisiae* (CAA85191) potential Slx1 sequences. Asterisks mark the cysteine and histidine residues conserved in the zinc-binding motifs.

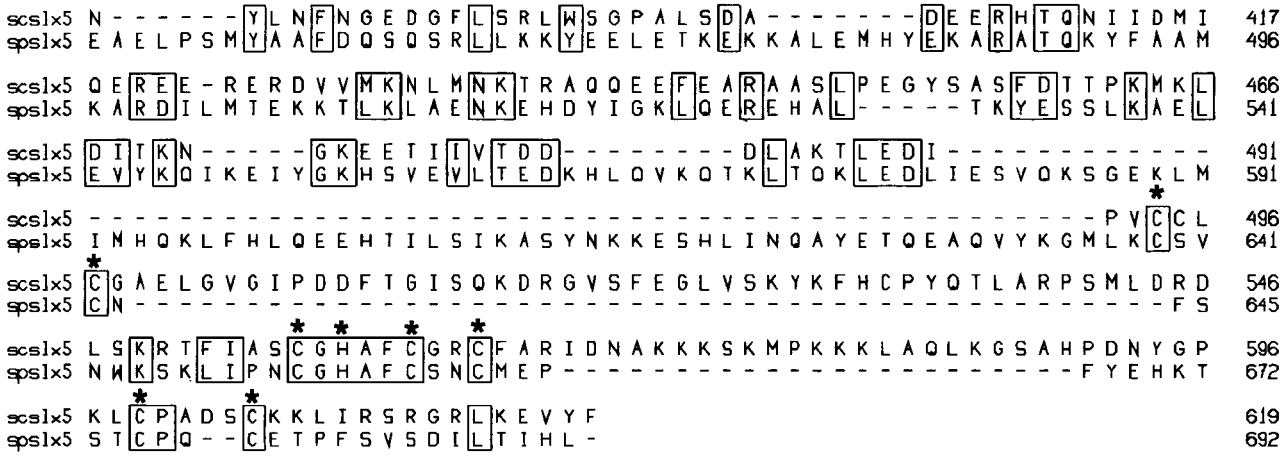
A

Slx5: C-X_2-C-$X_{12\text{-}58}$-C-X_1-H-X_2-C-X_2-C-$X_{11\text{-}34}$-C-$X_{2\text{-}4}$-C

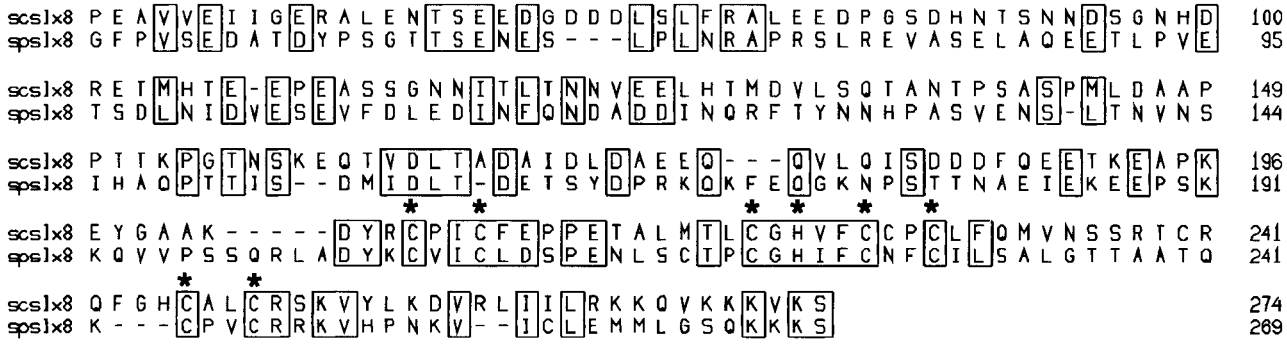
Slx8: C-X_2-C-X_{11}-C-X_1-H-X_2-C-X_2-C-$X_{13\text{-}16}$-C-X_2-C

Slx1: C-X_2-C-$X_{12\text{-}19}$-C-X_4-C-X_4-H-X_2-C-$X_{19\text{-}24}$-C-X_2-C

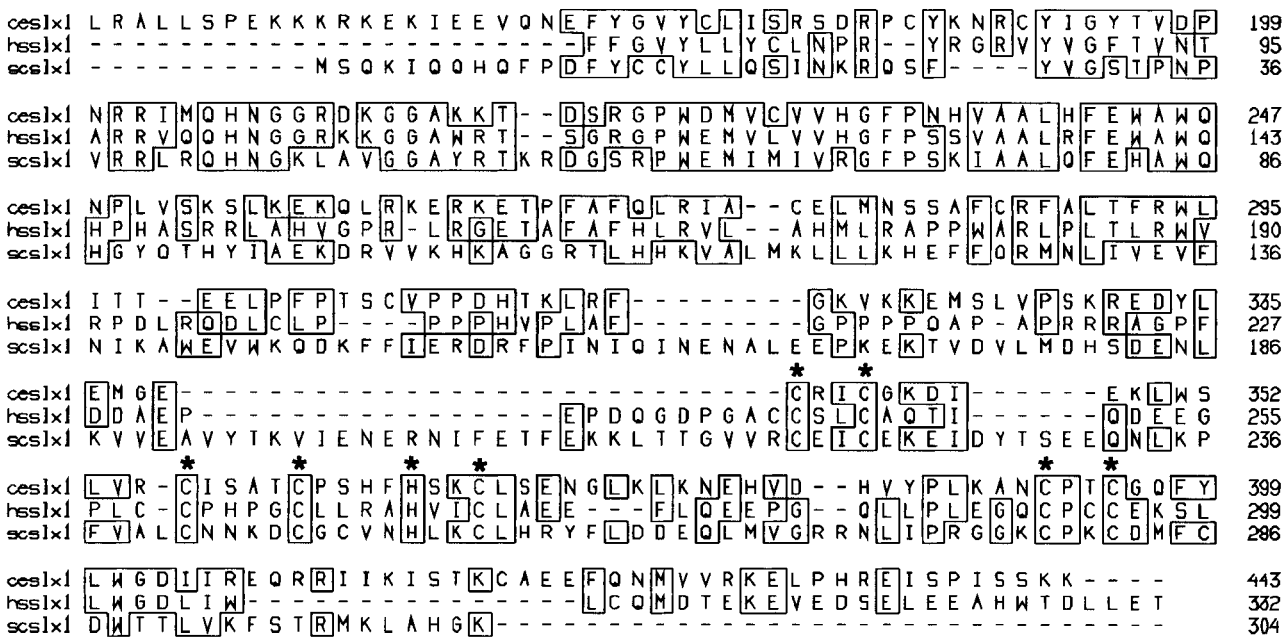
B



C



D



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 *recJ* 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 (Chakraverty 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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