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Sgs1: A Eukaryotic Homolog of E. coli RecQ That Interacts with Topoisomerase II In Vivo and Is Required for Faithful Chromosome Segregation

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Summary

Topoisomerase II (topo II) catalyzes the decatenation of interlinked DNA molecules and is essential for chromosome segregation. To test the hypothesis that the noncatalytic C-terminal domain of topo II is necessary for mediating interactions with other proteins required for chromosome segregation, we used a two-hybrid cloning strategy to identify proteins that interact with S. cerevisiae topo II in vivo. One protein identified (Sgs1p) is structurally related to E. coli RecQ protein and contains helicase signature motifs. Strains lacking Sgs1p exhibit elevated levels of chromosome missegregation during both mitotic and meiotic division. We propose a model to account for the interaction of a topoisomerase and a helicase in the faithful segregation of newly replicated eukaryotic chromosomes.

Introduction

The faithful segregation of chromosomes at mitosis and meiosis requires that the DNA is fully replicated, disentangled, and spatially separated in an ordered fashion. Errors in this process can lead to a failure to partition chromosomes equally during mitotic cell division, with the consequent production of daughter cells containing an abnormal complement of chromosomes. In the daughter cell that is hyperploid, gene dosage abnormalities may exist, while the daughter cell lacking a chromosome will be nonviable. Meiotic chromosome missegregation can have equally deleterious consequences, resulting either in sterile gametes or in aneuploid progeny.

Many proteins have been identified that influence the stability or segregation of chromosomes in eukaryotes. The majority of these affect either the synthesis of DNA (e.g., Cdc17p and Cdc9p; Hartwell and Smith, 1985) or the mechanics of chromosome separation (e.g., the Cin8 and Cin9 kinesin-like proteins; Samejima et al., 1993; Hoyt et al., 1992). A recently described gene family that includes the Saccharomyces cerevisiae *SMC1* gene, the Schizosaccharomyces pombe *cut3* and *cut14* genes, and the Xenopus laevis *XCAPC/E* genes appears to be involved directly in chromosome condensation, segregation, or both (reviewed by Peterson, 1994).

Another class of enzyme, in particular topoisomerase II (topo II), is required specifically for the resolution of interlinked chromosomes at mitosis (Holm et al., 1985, 1989; Uemura et al., 1987), as well as for decatenating the products of plasmid replication (DiNardo et al., 1984). In the absence of active topo II, mitotic chromosome segregation is still attempted, leading to chromosome breakage or nondisjunction, because the newly replicated chromosomes are still physically interlinked (reviewed by Wang, 1991; Holm, 1994; Watt and Hickson, 1994). Topo II is also required for meiotic division, and conditional *top2* mutants switched to the restrictive temperature arrest at the pachytene stage of meiosis I (Rose et al., 1990). However, unlike the situation in mitosis, chromosome segregation at meiosis I does not appear to be attempted in the absence of active topo II (Rose and Holm, 1993).

Residues in the C-terminal domain of S. cerevisiae topo II that are necessary for complementation of conditional *top2* alleles, yet are not required for enzymatic activity in vitro, have been identified recently (Caron et al., 1994). We hypothesized that the nonenzymatic function of the C-terminal domain of topo II might be to direct specific interactions with other proteins required to effect chromosome segregation. We sought, therefore, to identify proteins that can interact with the C-terminal region of topo II in vivo, using a two-hybrid cloning approach. We have identified a gene, designated *SGS1*, that has recently been identified independently by Gangloff et al. (1994) as a suppressor of the slow growth phenotype of *top3* (topoisomerase III) mutants. We show here that the SGS1 protein interacts specifically with a short region of the C-terminal domain that has previously been implicated in dimerization of the topo II protein itself. Strains lacking a functional *SGS1* gene show a reduced fidelity of both mitotic and meiotic chromosome segregation leading to a diminished capacity to undergo productive cell division.

Results

Cloning of the *SGS1* Gene via an Interaction with Topo II

To identify proteins that interact with the C-terminal domain of topo II, we employed a modification (Zervos et al., 1993) of the two-hybrid cloning strategy described by Fields and Song (1989). This strategy permits the detection of an interaction in vivo between an expressed bait protein (a DNA-binding domain fusion) and its interacting prey (a transcriptional activation domain fusion), which is present among a library of proteins coexpressed in yeast. In this instance, the bait protein consisted of the C-terminal domain (amino acids 1118–1429) of yeast topo II protein fused to the bacterial LexA protein (a construct designated pLexTopDT; see Figure 1).

A library of $>10^6$ independent transformants was recovered in the yeast strain EGY48 (Table 1) coexpressing the topo II bait protein. Out of ten clones that activated transcription in this screen, five were shown to represent overlapping sequences, confirming that the library coverage was adequate; one of these clones, designated

Table 1. Yeast Strains

Strain	Genotype	Synonym
EGY48[a]	*MATa, his3, trp1, ura3-52, lex(leu2)3a*	
CH1110[b]	*MATa, ade2-101, his3-Δ200, leu2-Δ1, lys2-801, trp1-Δ1, ura3-53, top2-4 [CF TRP⁺SUP11]*	
CH1110ΔSGS	*MATa, ade2-101, his3-Δ200, leu2-Δ1, lys2-801, trp1-Δ1, ura3-52, top2-4 [CF TRP⁺ SUP11], Δsgs1::LEU2*	
PW30	*MATα, ura3-n, leu2Δ, met13-2, cyh2*	D84-17c
PW30ΔSGS	*MATα, ura3-n, leu2Δ, met13-2, cyh2, Δsgs1::LEU2*	
PW40	*MATa, ura3-n, leu2Δ, met14-1, his6-1, lys9-1, trp1::URA3*	D90-6A
PW40ΔSGS	*MATa, ura3-n, leu2Δ, met14-1, his6-1, lys9-1, trp::URA3, Δsgs1::LEU2*	
PW50	*MATα, ura3-52 leu2Δ, ade2-101, lys2, cyh2*	EJL374-12D
PW50ΔSGS	*MATα, ura3-52 leu2Δ, ade2-101, lys2, cyh2, Δsgs1::LEU2*	
YPH277[c]	*MATa, ura3-52, lys2-801, ade2-101, trp1Δ1, leu2Δ1, CRVII (RAD2.d.YPH277) URA3 SUP11*	
YPΔRQH	*MATa, ura3-52, lys2-801, ade2-101, trp1Δ1, leu2Δ1, CRVII (RAD2.d.YPH277) URA3 SUP11, Δsgs1::LEU2*	
PWD60	*MATa, ura3-52, lys2-801, ade2-101, trp1Δ1, leu2Δ1, CRVII (RAD2.d.YPH277) URA3 SUP11* / *MATα, ura3-52 leu2Δ, ade2-101, lys2, cyh2*	
PWD60Δ	*MATa, ura3-52, lys2-801, ade2-101, trp1Δ1, leu2Δ1, CRVII (RAD2.d.YPH277) URA3 SUP11, Δsgs1::LEU2* / *MATα, ura3-52 leu2Δ, ade2-101, lys2, cyh2, Δsgs1::LEU2*	
PWD70	*MATα, ura3-n, leu2Δ, met13-2, cyh2* / *MATa, ura3-n, leu2Δ, met14-1, his 6-1, lys 9-1, trp1::URA3*	
PWD70Δ	*MATα, ura3-n, leu2Δ, met13-2, cyh2, Δsgs1::LEU2* / *MATa, ura3-n, leu2Δ, met14-1, his 6-1, lys 9-1, trp1::URA3, Δsgs1::LEU2*	

PWD60 was made by mating the YPH277 and PW50 haploid strains. PWD70 was made by mating the PW30 and PW40 haploid strains. Diploids PWD60Δ and PWD70Δ are isogenic homozygous deletions of the *SGS1* gene in the PWD60 and PWD70 strains, respectively, made by mating the corresponding *Δsgs1* haploids.

[a] Gyuris et al., 1993.

[b] Supplied by C. Holm.

[c] Spencer et al., 1990.

pActSGS1 (Figure 1), was taken for further study. The predicted amino acid sequence derived from the only long open reading frame within this clone is identical to the recently identified Sgs1 protein (Gangloff et al., 1994) and shows strong homology to the sequence of the E. coli RecQ protein (see below).

Sgs1p Interacts with the Putative Leucine Zipper Region of Topo II

To determine the region of topo II with which Sgs1p interacts, the activation assay was repeated using LexA fusions other than pLexTopDT. The pActSGS1 construct failed to activate transcription in the presence of a topo II fusion containing the extreme C-terminal tail comprising amino acid residues 1168–1429 (pLexTopT; Figure 1), but did activate transcription with a construct containing only the putative leucine zipper region (pLexTopD, containing residues 1109–1163; Figure 1). pActSGS1 failed to activate transcription significantly in the presence of two nonspecific LexA fusions, pHM12 and pLex-MAX (Figure 1), indicating that the interaction with topo II was apparently specific.

Sgs1p Interacts with Functional Topo II

The possibility of an artifactual interaction between Sgs1p and the C-terminal domain of topo II, due to some aspect of the two-hybrid system, could not be discounted. Therefore, we tested whether Sgs1p could interact with catalytically active topo II protein in vivo. Sgs1p tagged with a hemagglutinin (HA) epitope was expressed in EGY48 together with a construct (pTopoII-myc) expressing Myc-tagged topo II (Lindsley and Wang, 1991; see Figure 1). Figure 2 shows that the HA-tagged Sgs1p coimmunoprecipitated with topo II, while a nonspecific monoclonal antibody of the same subclass failed to immunoprecipitate Sgs1p from the same extract.

Sgs1p Is a Yeast Homolog of E. coli RecQ

The pActSGS1 activation clone was clearly incomplete, and thus we screened a S. cerevisiae λgt11 cDNA library using sequences derived from pActSGS1. The longest cDNA isolated (pSGS1 cDNA of 1.4 kb) contained a continuous open reading frame that was identical to an amino acid sequence that had been submitted to the SwissProt data base under the name TPS1 (accession number P35187), as well as to the recently published sequence of the *SGS1* gene product (Gangloff et al., 1994; GenBank accession number U22341). Comparison of the predicted Sgs1 amino acid sequence with sequences in the Swissprot database revealed extensive similarity (Figure 3) to the RecQ protein, a DNA helicase involved in the RecF pathway of genetic recombination in E. coli *SGS* (Luisi-DeLuca et al., 1989; Umezu et al., 1990). Over a 468 amino acid stretch, 38% of the residues of Sgs1p and the RecQ protein are identical and 71% are similar (if conservative changes are included). Notable sequence features include a putative ATP-binding site and several of the motifs commonly found in RNA or DNA helicases, including the DEAH box (Gorbalenya et al., 1989; Koonin, 1991). The most conserved regions are represented by these helicase motifs (Figure 3). Despite the strong similarity between the two proteins, the yeast enzyme is predicted

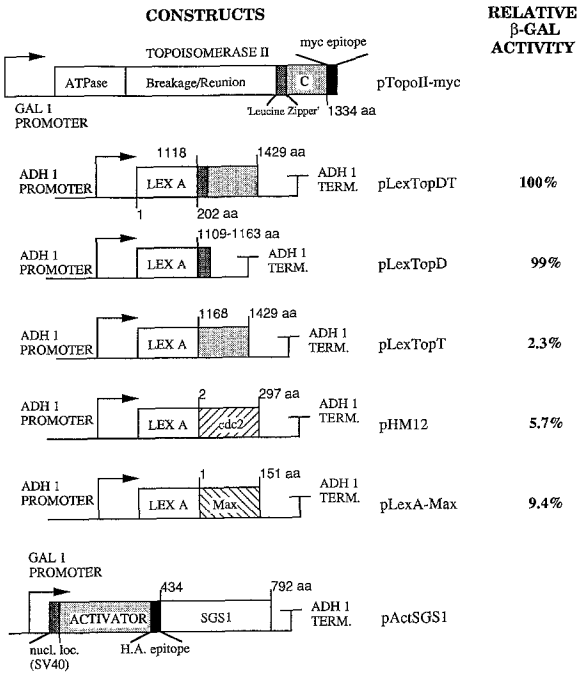

Figure 1. Plasmids Used in This Study

Schematic diagram (not to scale) of relevant regions of constructs used. The leucine zipper and C-terminal tail (C) of topo II are indicated. Constructs pLexTopDT, pLexTopD, and pLexTopT contain the specified regions (amino acid numbers are shown) of topo II fused to the full coding sequence of LexA. pHM12 and pLexA-Max are nonspecific LexA fusions of a Drosophila Cdc2 kinase homolog and human MAX protein, respectively. pActSGS1 is the activation clone isolated in the two-hybrid screen and includes an HA epitope tag (YPYDVPDYA). The column on the right shows relative β-galactosidase activity, expressed as a percentage of the most strongly activating construct (pLexTopDT).

to be considerably larger (1447 amino acids) than the bacterial enzyme (607 amino acids). The region of Sgs1p that includes the domain of interaction with topo II is indicated in Figure 3. It is perhaps significant that this region con-

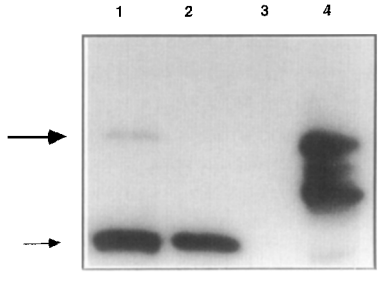

Figure 2. Coimmunoprecipitation of Sgs1p and Topo II

An autoradiograph of a Western blot with anti-HA antibody of proteins immunoprecipitated using anti-Myc (lane 1) or anti-ICAM1 antibody (lane 2). In both cases, extracts were prepared from EGY48 harboring pTopII-myc and pActSGS1. Lane 3 was left blank. Lane 4 contains an extract from EGY48 harboring pActSGS1. The full-length ActSgs1 fusion protein runs as the upper band at approximately 60 kDa and is indicated by the large arrow. The small arrow indicates the position of the immunoglobulin G heavy chain (approximately 50 kDa) and serves as an internal loading control.

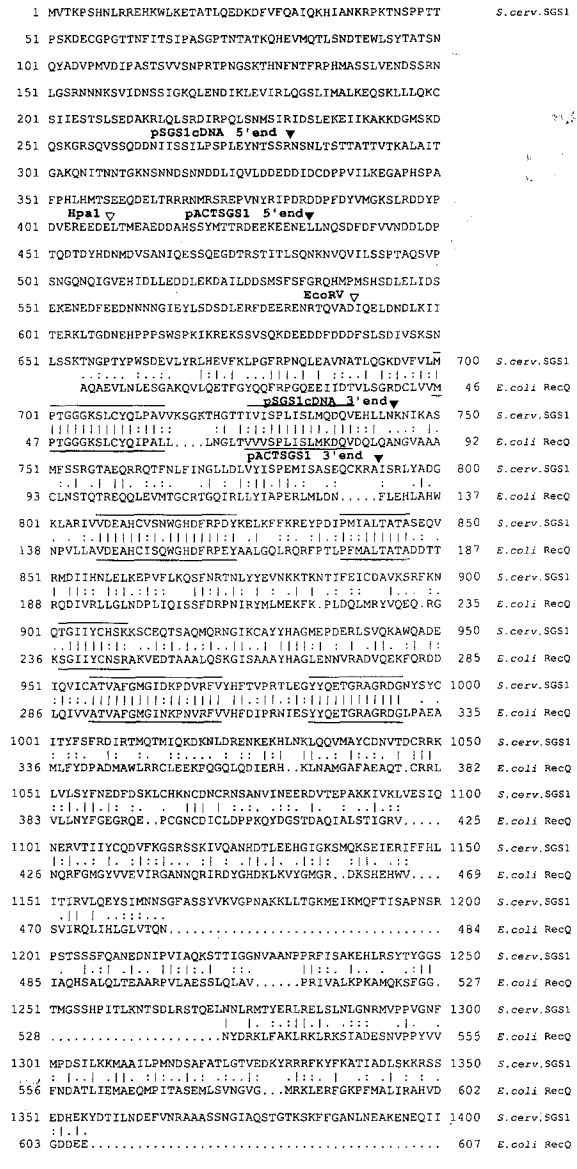

Figure 3. Comparison of the Sequences of Sgs1p and the RecQ Protein

The predicted amino acid sequence of a portion of Sgs1p from residue 1 to the end of the region of homology with E. coli RecQ protein is aligned with the RecQ protein sequence. Identical residues are indicated by vertical lines. Conserved residues are indicated by dots. The regions of maximal conservation represented by the seven helicase motifs are overlined and underlined. These motifs include the putative ATP-binding site (the first highlighted domain) and the DEAH box (the third highlighted domain). The 5′ and 3′ ends of the pActSGS1 and pSGS1 cDNA clones are indicated by closed inverted triangles. The positions of the HpaI and EcoRV sites that flank the region deleted in the *Δsgs1* strains are indicated by open inverted triangles.

tains two of the highly conserved sequence motifs (MPTGGGKSL and TIVISPLPE). The N-terminal region of Sgs1p is not conserved in RecQ.

Deletion of the *SGS1* Gene Affects Cell Growth

To investigate the function of Sgs1p, we generated targeted deletions of the *SGS1* gene in several strains. The

Table 2. Mitotic Chromosome Missegregation in *SGS1* and *Δsgs1* Strains

Strain	Frequency Class 1[a] (%)	Frequency Class 2[b] (%)	Frequency Class 3[c] (%)	Frequency Class 4[d] (%)	Ratio Nondisjunction Loss[e]
PWD60	0.02	0.00	0.02	0.02	0.50
PWD60Δ	0.23	0.48	0.05	0.23	2.54

[a] Class 1 represents 1:1 red:white sectored colonies (nondisjunction).
[b] Class 2 represents 1:1:2 red:white:pink sectored colonies (nondisjunction).
[c] Class 3 represents 1:1 red:pink sectored colonies (chromosome loss).
[d] Class 4 represents 1:3 red:pink sectored colonies (chromosome loss).
[e] Calculated by $\frac{\text{Class 1} + \text{Class 2}}{\text{Class 3} + \text{Class 4}}$.

construct used to make the deletions contains the *LEU2* gene inserted after codon 408 and includes a deletion of 540 bp of the *SGS1* coding sequence, removing all highly conserved domains including the predicted ATP-binding site (Figure 3). The possibility of downstream readthrough producing a Leu2–Sgs1 fusion protein can be eliminated, owing to the presence of intervening termination codons. The *sgs1* deletion strains (designated by the suffix *Δsgs1*; Table 1) grew more slowly than isogenic parental controls, producing significantly smaller colonies on solid medium. This reduced growth rate was at least partly dependent upon a reduction in mutant cell viability (data not shown).

Sporulation of *Δsgs1* Diploids Results in Reduced Meiotic Viability

Dissection of tetrads derived from sporulation of the homozygous *sgs1* deletion strain PWD80Δ revealed that both tetrad formation and spore viability were markedly reduced when compared with the isogenic control PWD80 strain. Out of 40 asci analyzed from PWD80, overall spore viability was 98%, and >90% of the asci contained four viable spores. In contrast, out of 224 asci from PWD80Δ, overall spore viability was only 71%, with 35% of the total being represented by the two or fewer viable spore class and 27.5% by the three viable spore class. This decrease in spore viability was observed with two independently constructed *Δsgs1* diploids made from distinct *Δsgs1* haploid mutants.

Deletion of the *SGS1* Gene Causes Increased Mitotic Chromosome Nondisjunction

Since the phenotype of *sgs1* strains could be explained by a stochastic chromosome segregation defect, we made homozygous deletions of the *SGS1* genes in the diploid strain PWD60. This strain carries one copy of a chromosomal fragment marked with an ochre suppressor (*SUP11*) of an *ade2* mutation (Spencer et al., 1990). In PWD60, chromosome loss (1:0 segregation) can be distinguished from chromosome nondisjunction (2:0 segregation) by the color of the sectors that arise in colonies (see Experimental Procedures). The results (Table 2) show that there was a greater than 10-fold increase in the frequency of chromosome missegregation in the *sgs1* mutant strain PWD60Δ when compared with the isogenic wild-type control PWD60. Moreover, these data demonstrated that 72% of the mitotic missegregation events observed in PWD60Δ could be attributed to chromosome nondisjunction, rather than chromosome loss.

Sgs1p and Topo II Appear to Act in the Same Chromosome Segregation Pathway

To confirm that *Δsgs1* strains exhibit mitotic chromosome missegregation and to address whether the *Δsgs1* mutation is epistatic to a *top2-4* mutation, we deleted the *SGS1* gene in CH1110, a strain constructed specifically for an analysis of chromosome missegregation in a conditional *top2* background (Holm et al., 1989). In this strain, missegregation is measured as before by the rate of loss of a chromosomal fragment, although chomosome loss cannot be distinguished from nondisjunction. Deletion of the *SGS1* gene in this strain caused an increase in the level of red sectoring, consistent with a stimulation in missegregation. Fluctuation analyses (Luria and Delbruck, 1943) indicated that missegregation was elevated at least 10-fold in *Δsgs1* derivatives grown at 25°C, the permissive temperature for *top2-4* (Table 3). When these analyses were repeated at 30°C, the semipermissive temperature for CH1110, the rate of missegregation increased approximately 10-fold in the SGS1$^+$ strain, owing to the partial loss of topo II function. However, the presence of an *sgs1* mutation did not significantly influence this elevated rate at the semipermissive temperature (Table 3), indicating that Sgs1p and topo II are likely to function in the same segregation pathway.

An *sgs1* Deletion Causes a High Level of Missegregation at Meiosis I

The high level of spore nonviability in the *sgs1* mutant suggested a defect either in sporulation or in the process of chromosome segregation during meiosis. We tested the latter possibility by introducing the *Δsgs1* mutation into strain PWD70. This strain is marked genetically for the specific detection of meiosis I missegregation (Louis and Haber, 1989). One of the haploid parents of PWD70 has both a *URA3* disruption of the *TRP1* gene adjacent to the centromere on chromosome IV and a stable *ura3* mutation. The other haploid parent is wild type for *TRP1*, but carries the same *ura3* mutation. Since *trp1::URA3* and *TRP1* are allelic in the diploid strain, crossing over between the two markers is not possible. One haploid parent of the diploid carries a recessive mutation conferring resistance to cyclohexamide (*cyh2*). Therefore, the only means of

Table 3. Epistasis of *top2* and *sgs1* Mutations

Strain	Growth Temperature (in Celsius)	Total Colonies Counted	Total Frequency *ade*⁻ (%)	Median Frequency *ade*⁻ (%)	Loss Rate per Generation × 10^{-4}
CH1110	25	2625	0.11[a]	Undetectable[b]	–
CH1110ΔSGS	25	2105	1.00	0.77	2.2
CH1110	30	847	1.06	1.30	4.2
CH1110ΔSGS	30	1362	1.17	1.26	3.7

[a] The total *ade*⁻ CH1110 colonies, divided by the total CH1110 colonies counted, gave the value 0.11%, which is likely to be an overestimate of the background frequency due to bias resulting from so-called jackpot events.
[b] In most determinations (71%), no *ade*⁻ colonies were detected, precluding the calculation of a median.

generating spores that are simultaneously cyclohexamide resistant (Cyh^r), Ura$^+$, and Trp$^+$ is via missegregation of chromosome IV during meiosis I. Deletion of *SGS1* in PWD70 causes an average 37-fold stimulation in the degree of meiosis I missegregation of chromosome IV (Table 4). The background level of meiotic missegregation of chromosome IV in this strain is similar to that observed in other backgrounds (Louis and Haber, 1989).

Discussion

A truncated topo II enzyme that lacks the C-terminal 263 amino acids failed both to decatenate DNA in vivo and to complement the mitotic nondisjunction defect in a *top2-4* strain at the restrictive temperature. Paradoxically, the same mutant enzyme exhibited full DNA relaxation and decatenation activity in vitro (Caron et al., 1994). We tested the hypothesis that the C-terminal domain of topo II is required for efficient chromosome segregation in vivo owing to its involvement in directing interactions with other proteins in the nucleus. Using a yeast two-hybrid activation library, we cloned the *SGS1* gene through its interaction in vivo with the C-terminal domain of topo II. The *SGS1* gene encodes a protein with strong primary sequence similarity to the E. coli RecQ helicase. However, the predicted size of the Sgs1 protein (called Sgs1p) is considerably larger than that of RecQ, implying that Sgs1p might carry out functions unrelated to those performed by RecQ in E. coli. Nevertheless, the high degree of conservation between these E. coli and S. cerevisiae proteins suggested the possibility of finding other homologs of Sgs1p in higher eukaryotes. Indeed, related sequences in Caenorhabditis elegans (GenBank accession number U00052) and human (Seki et al., 1994; Puranam and Blackshear, 1994) have been identified recently, suggesting that the RecQ-like proteins are widely conserved in evolution.

Chromosome Missegregation in *Δsgs1* Mutants

We have presented evidence for a significant elevation in the rate of chromosome nondisjunction during mitotic division in *Δsgs1* strains. The finding that the stimulation of chromosome nondisjunction caused by deletion of *SGS1* never exceeded that caused by a mutation in *TOP2* when studied at the semipermissive temperature indicates that the products of the *TOP2* and *SGS1* genes are likely to be acting in the same segregation pathway.

Deletion of the *SGS1* gene also causes a substantial stimulation of meiotic missegregation. The assay that we used specifically measures aneuploids produced at meiosis I (i.e., meiosis I nondisjunction or precocious sister segregation) rather than those produced in meiosis II (i.e., meiosis II nondisjunction). If the spore nonviability observed was indeed due to nondisjunction in meiosis I, it is expected that a disproportionate representation of the two or fewer viable spore class would be obtained. This was what was found. We also observed some increase in the three viable spore class, suggesting that meiosis II nondisjunction or precocious sister segregation at meiosis I could also be occurring at some frequency in *Δsgs1* strains. Analysis of the segregation of markers in the progeny of dissected asci suggests that precocious sister segregation is contributing to the observed meiosis I missegregation in *Δsgs1* mutants (data not shown). Considering the effects of an *SGS1* deletion on mitotic nondisjunction,

Table 4. Meiosis I Missegregation

Strain	Cyh^r Ura$^+$ Trp$^+$ Prototrophs	Cyh^r Colony-Forming Units	Meiotic Frequency Ura$^+$ Trp$^+$ (%)	Fold Increase[a] Ura$^+$ Trp$^+$
PWD70	1.4×10^2	2.4×10^5	0.06	1
PWD70Δ	1.7×10^4	1.6×10^6	1.04	18
PWD70Δ	2.7×10^5	5.3×10^6	4.26	72
PWD70Δ	1.1×10^6	5.7×10^7	1.92	32
PWD70Δ	2.2×10^5	1.6×10^7	1.36	23
PWD70Δ	7.2×10^5	3.2×10^7	2.22	38

Data derived from the parental diploid (PWD70) and five independent mutant diploid strains (PWD70Δ) are indicated.
[a] Fold increase in *sgs1* strain compared with wild type.

we would suggest that it is also likely that nondisjunction is also occurring at some frequency during meiosis II because of the mechanistic similarity between meiosis II and mitosis.

Role of Topo II in Chromosome Segregation

In yeast, the predominant pathway for replication results in the newly replicated chromosomes being intertwined (reviewed by Murray and Szostak, 1985; Wang, 1991; Holm, 1994), as also appears to be the case for bacterial plasmid DNA (Adams et al., 1992). Topo II is required for the decatenation of interlinked DNA molecules that accumulate as a result of DNA replication (Sundin and Varshavsky, 1981; see model below). Thus, in the absence of a decatenase activity at mitosis in yeast, chromosomes can fragment and nondisjoin, presumably owing to a failure to remove covalently closed interlocks during or prior to anaphase (Holm et al., 1989).

Model for an Interaction of a Topoisomerase and a Helicase in Chromosome Segregation

If Sgs1p is a DNA helicase, as seems highly likely based upon its homology to a family of DNA helicases, why should it associate with topo II, and what role might this interaction play in chromosome segregation? Clearly, the interaction of Sgs1p and topo II could involve an active coordination of the enzymatic functions of the two enzymes. Precedent for the functional association of a helicase and a topoisomerase activity is found in the archebacterial "reverse gyrase" enzyme (Confalonieri et al., 1993). In this case, the combination of helicase and topo I domains in the same polypeptide results in an enzyme that not only unwinds DNA, but also introduces positive supercoils into it. It is possible that the interaction between Sgs1p and a topoisomerase fulfills this function in yeast cells for the purpose of manipulating the degree of supercoiling of cellular DNA.

On the basis of the observed missegregation phenotype of an *sgs1* mutant, we suggest that it is more likely that the association of Sgs1p and topo II serves to facilitate the segregation of newly replicated chromosomes. We propose the following model to explain the functional association of a helicase and a topoisomerase in eukaryotic cells. At the terminal stages of the replication of a circular DNA molecule or a topologically constrained domain of a linear chromosome, significant steric constraints preclude topoisomerase enzymes from accessing the region of converging replication forks owing to a build up of DNA supercoils. One solution to this problem is for a DNA helicase (proposed to be Sgs1p) to unwind the duplex, thereby converting the supercoiled DNA into interlocked catenanes. The short region of single-stranded DNA thus produced can then be fully replicated, prior to decatenation of the sister molecules by topo II. A helicase activity has likewise been invoked in the two-stage model for the unlinking of plasmid catenanes in bacteria (Adams et al., 1992). In the absence of a helicase, not all of the DNA molecules would necessarily complete replication, resulting in interconnected sister chromatids and consequent nondisjunction. Topo II presumably only has a limited time window in which to catalyze the removal of chromosomal interlocks generated by replication, since no mitotic checkpoint arrest occurs in response to a failure to decatenate DNA in budding yeast and since *top2* mutants still attempt to segregate interlocked chromosomes.

Although the functional role of topo III has yet to be established, it is of interest that Sgs1 protein is known to interact in vivo with both topo II (this work) and topo III (Gangloff et al., 1994). Whether the interaction of Sgs1 with two distinct topoisomerases in S. cerevisiae implicates these three proteins in forming a multienzyme complex required for chromosome segregation will require further investigation.

The proposed model could also account for meiosis I nondisjunction, if one postulates that Sgs1p is required to prevent entanglement resulting from the final stages of sister chromatid replication prior to meiosis I. If the sister chromatids are still unresolved when the synaptonemal complex forms, reciprocal recombination would generate entangled homologous chromosomes that would not be segregated evenly. It may be the case that some degree of redundancy exists in yeast for the resolution of replication intermediates and that a less efficient parallel system is sufficient in many cases to permit continued cell division in the absence of Sgs1p. However, it is likely that alternative pathways would require functional topo II in yeast.

In work to be published elsewhere, we will show that *sgs1* strains exhibit mitotic hyperrecombination. Hartwell and Smith (1985) have suggested that mutations that stimulate mitotic recombination as well as chromosome instability are likely to have defects in DNA metabolism, rather than in the segregation process per se. However, an important exception is a mutation in the *TOP2* gene itself, which confers hyperrecombination (particularly at the rDNA locus) as well as a defect in chromosome segregation. In general, mutations in genes that influence recombination, such as those of the *RAD52* epistasis group, lead to nonreciprocal chromosome transmission abnormalities, primarily chromosome loss, owing to a failure to repair damaged chromosomes (Mortimer et al., 1981). In contrast, an *sgs1* mutant resembles a *top2* mutant in showing hyperrecombination at the rDNA locus and a chromosome transmission abnormality that is primarily reciprocal in nature (i.e., nondisjunction). Moreover, a mutation in *RAD52* is neither synthetically lethal with an *sgs1* mutation, nor able to suppress the elevated frequency of nondisjunction conferred by an *sgs1* mutation (unpublished data). These data suggest that cytotoxic DNA lesions, such as unrepaired double-stranded breaks, do not persist in *sgs1* strains and that the abnormal chromosome segregation phenotype of an *sgs1* mutant is unlikely to be a direct consequence of an elevation in the rate of recombination.

Little is known about the genetic basis for nondisjunction in humans, although this appears to be a surprisingly common event in human meioses. In a recent karyotype of 185 human oocytes, 11% were shown to be aneuploid, and it was suggested that meiosis I nondisjunction was likely to be the major factor contributing to this aneuploidy (Kamaguchi et al., 1993). It is hoped that as the molecular mechanisms of chromosome segregation become clearer,

our knowledge of the causes of nondisjunction will increase. Such information may aid in the elucidation of the underlying factors responsible for human disease conditions such as Down's syndrome that are caused by karyotypic instability.

Experimental Procedures

S. cerevisiae Strains

The genotypes of strains used in this work are listed in Table 1. Strain EGY48 (Gyuris et al., 1993) was obtained from Dr. R. Brent. Strain YPH277 (Spencer et al., 1990) was obtained from Dr. P. Heiter. PW30 (D84-17c) and PW40 (D90-6A) are isogenic derivatives of Y55 (McCusker and Haber, 1988) genetically marked to assay meiosis I missegregation events (Louis and Haber, 1989). Strain CH1110 was obtained from Dr. C. Holm. Strain PW50 (EJL374-12D) is an isogenic derivative of YP1 (Louis and Haber, 1989). Isogenic deletions of the *SGS1* gene in the above strains were made by transformation (Ausubel et al., 1994) with pPWΔSGS1 that had been digested with NcoI and PstI. Diploid strains were constructed by mating followed by appropriate selection, as well as confirmation of nonmating phenotype.

Growth of Microorganisms and DNA Manipulation

E. coli and yeast growth and standard recombinant DNA techniques were as described by Ausubel et al. (1994) and Sherman (1991).

Plasmids

The plasmid pJG45 (Gyuris et al., 1993), in which the activation library was constructed (see below), together with the LexA–fusion DNA-binding domain vector (pEG202) and the *lacZ* reporter plasmid (pSH1834), was provided by Dr. R. Brent. pLexTopDT was constructed by cloning an SspI fragment of *TOP2* (representing residues 1118–1429) in-frame with *lexA* into pEG202. pLexTopD and pLexTopT constructs (which contain residues 1109–1163 and 1168–1429, respectively) were made similarly, except that in the construction of pLexTopT, an octameric EcoRI linker was inserted into the EcoRV site of *TOP2* to create the in-frame 5′ cloning junction. The nonspecific LexA fusion plasmids pLexA-Max (Zervos et al., 1993) and pHM12 (a gift from Drs. R. Finley and R. Brent) contain the entire coding region of the human MAX protein and 295 residues of Drosophila melanogaster Cdc2 kinase, respectively.

To generate pPWΔSGS1 for targeted disruption of *SGS1*, the 5′ end of *SGS1* (corresponding to amino acid residues 1–792) was amplified from genomic S. cerevisiae DNA (S288C; ATCC number 26108) by polymerase chain reaction and cloned between the BamHI and Asp-718 sites of vector pQE32 (Qiagen). The resulting construct, pPWQ3, was then digested with HpaI and EcoRV (deleting 540 bp of *SGS1* sequence), prior to the insertion of a 2230 bp fragment containing the *LEU2* gene to generate pPWΔSGS1.

Activator Fusion Library Construction

Genomic DNA from a diploid S. cerevisiae S288C strain (ATCC number 26108) was divided into two pools. One pool was partially digested with AluI, while the other pool was partially digested with HaeIII, to yield in each case a median fragment size of approximately 1 kb. Each pool was treated with EcoRI methylase, and the extent of methylation was determined by trial digestion with EcoRI. An equimolar mixture of the EcoRI linkers d(pGGAATTCC), d(pCGGAATTCCG), and d(pCCGGAATTCCGG) was ligated onto each of the methylase-treated pools before digesting the ligation products with EcoRI. The DNA was then size fractionated (to between 0.8 and 4 kb) using agarose gel electrophoresis. The two purified preparations were then pooled and cloned into the pJG45 activator fusion vector digested with EcoRI and dephosphorylated. The ligated products were transformed into E. coli to yield 3×10^6 to 5×10^6 primary transformants. Plasmid DNA was prepared directly from the pooled, unamplified E. coli transformants.

Two-Hybrid Screen

The two-hybrid screen was performed essentially as described by Gyuris et al. (1993) and Zervos et al. (1993). The yeast activation domain library described above was transformed into the reporter strain EGY48, which had previously been transformed with pLexTopDT and pSH1834. Plasmids were isolated from yeast that survived selection for leucine prototrophy and were blue on X-Gal-containing plates. Quantification of β-galactosidase activity was performed as described by Ausubel et al. (1994) using extracts, normalized for total protein, prepared from exponentially growing yeast treated with 2% galactose for 4 hr.

Immunoprecipitations

These were performed essentially as described by Kolodziej and Young (1991). Strain EGY48 was transformed with plasmids pActSGS1 (encoding HA-tagged Sgs1p) and pTopoII-myc (encoding Myc-tagged topo II; Lindsley and Wang, 1991). Yeast were lysed, and immunoprecipitations were carried out using either anti-Myc (9E10) antibody or an anti-ICAM1 control antibody of the same subclass (IgG1). Western blots were probed with the 12CA5 anti-HA monoclonal antibody, using standard techniques (Ausubel et al., 1994).

Mitotic Missegregation

Control strain CH1110 and the isogenic *sgs1* mutant strain (CH1110ΔSGS) contain a *TRP1*-marked chromosome fragment bearing the *SUP11* suppressor gene of an ochre *ade2-101* mutation. The presence of the suppressor allele results in a white colonies, while its absence results in red colonies. Following selection on SC medium lacking tryptophan at 25°C, entire single colonies were picked and replated on nonselective yeast extract–peptone–dextrose (YPD) agar at either 25°C (permissive for *top2-4*) or 30°C (semipermissive). The resultant white or sectored colonies were then replated onto YPD agar. The number of pure red colonies as a fraction of the total was determined. Fluctuation analysis was performed as described by Lea and Coulson (1949) using the method of the median.

A similar analysis was carried out in the diploid strain PWD60 to distinguish chromosome nondisjunction from chromosome loss (Spencer et al., 1990). This *ade2-101* strain contains the *SUP11* gene on a single copy of a *URA3*-marked chromosomal fragment. A dosage of one copy of *SUP11* renders this strain pink on low adenine SC medium. *SUP11* dosages of 2 and 0, resulting from nondisjunction of the chromosomal fragment, produce adjacent white and red sectors, while chromosome loss results in a red sector without an adjacent white sector. Colonies with half sectors (red:white or red:pink) and quarter sectors (¼ red:¼ white:½ pink or ½ red:¾ pink) were scored. The frequency of missegregation events is represented by the fraction of half-sectored colonies to the total number of cells plated (the event occurred in the first mitotic division) and the fraction of quarter-sectored colonies out of twice the number of cells plated (the event occurred after one mitotic division).

Meiosis I Missegregation

Diploids were selected on SC medium lacking methionine, and single colonies were used to prepare patches on a nonselective YPD plate. Yeast were replica plated onto sporulation medium, and the plates were incubated at 20°C for 5 days. Gluculase (New England Nuclear)-treated spores were sonicated briefly before plating dilutions both on SC medium and on SC medium lacking Ura and Trp, in each case containing Cyh at 10 μg/ml. The frequency of meiosis I missegregation was calculated as the ratio of total (Cyh^r Ura^+ Trp^+) spores to total Cyh^r spores.

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