Centromere-Proximal Crossovers Are Associated With Precocious Separation of Sister Chromatids During Meiosis in *Saccharomyces cerevisiae*

Beth Rockmill,* Karen Voelkel-Meiman* and G. Shirleen Roeder*,†,1

**Howard Hughes Medical Institute, Department of Molecular, Cellular and Developmental Biology and
†Department of Genetics, Yale University, New Haven, Connecticut 06520-8103*

ABSTRACT

In most organisms, meiotic chromosome segregation is dependent on crossovers (COs), which enable pairs of homologous chromosomes to segregate to opposite poles at meiosis I. In mammals, the majority of meiotic chromosome segregation errors result from a lack of COs between homologs. Observations in *Homo sapiens* and *Drosophila melanogaster* have revealed a second class of exceptional events in which a CO occurred near the centromere of the missegregated chromosome. We show that in wild-type strains of *Saccharomyces cerevisiae*, most spore inviability is due to precocious separation of sister chromatids (PSSC) and that PSSC is often associated with centromere-proximal crossing over. COs, as opposed to nonreciprocal recombination events (NCOs), are preferentially associated with missegregation. Strains mutant for the RecQ homolog, *SGS1*, display reduced spore viability and increased crossing over. Much of the spore inviability in *sgs1* results from PSSC, and these events are often associated with centromere-proximal COs, just as in wild type. When crossing over in *sgs1* is reduced by the introduction of a nonnull allele of *SPO11*, spore viability is improved, suggesting that the increased PSSC is due to increased crossing over. We present a model for PSSC in which a centromere-proximal CO promotes local loss of sister-chromatid cohesion.

THE first division of meiosis is distinct from mitosis and meiosis II in that sister chromatids remain associated with each other, while homologous chromosomes segregate to opposite spindle poles. Proper chromosome segregation at meiosis I depends on crossing over, which establishes chromatin bridges (called chiasmata) between homologs (for review, see PAGE and HAWLEY 2003; PETRONCZKI *et al.* 2005). During prometaphase, homologs can become attached to microtubules from the same or opposite spindle poles. Only attachment to opposite poles results in a stable configuration that is maintained until anaphase. Recognition that chromosomes are properly oriented depends on the mechanical tension that results when homologs are pulled toward opposite spindle poles, and this pulling is resisted by chiasmata. Homologs are held together by chiasmata because sister chromatids are glued to each other in regions distal to chiasmata by sister-chromatid cohesion. Cohesion distal to chiasmata is released at anaphase I, as chromosomes begin their poleward movement. Cohesion near the centromere persists until anaphase II when sister chromatids separate and segregate.

The major cause of aneuploid gametes in mammals and Drosophila is a failure to cross over, and thus failure to form a chiasma, between a pair of homologous chromosomes (MACDONALD *et al.* 1994; HASSOLD *et al.* 1996; KOEHLER *et al.* 1996; LAMB *et al.* 1996). Failure to cross over results in random segregation, such that homologs are just as likely to move to the same pole as to opposite poles at anaphase I. When nondisjunction at meiosis I is followed by equational chromosome segregation at meiosis II, two of the resulting products lack a copy of the missegregated chromosome and two are disomic, carrying one chromatid from each homolog [referred to as a meiosis I (MI) disome]. These types of nondisjunction events are extremely rare in wild-type budding yeast, presumably because this organism undergoes high levels of meiotic recombination. However, in yeast mutants with reduced levels of crossing over, these events are common (ROSS-MACDONALD and ROEDER 1994; SYM and ROEDER 1994). In addition, yeast artificial chromosomes frequently undergo meiosis I nondisjunction because these chromosomes do not recombine as efficiently as normal chromosomes (ROSS *et al.* 1996).

A second type of meiotic missegregation observed in both mammals and Drosophila leads to disomes containing both chromatids from the same homolog (MII disomes). This type of gamete represents ~22% of aberrant segregation events in humans and ~6% in flies (KOEHLER *et al.* 1996; LAMB *et al.* 1996; HASSOLD and HUNT 2001). Rather than being nonrecombinant, these disomes are associated with crossovers (COs) specifically near the centromere. Although the missegregation event occurs at meiosis II, the predisposing event, a

[1] *Corresponding author:* Howard Hughes Medical Institute, Department of Molecular, Cellular and Developmental Biology, Yale University, PO Box 208103, 266 Whitney Ave., New Haven, CT 06520-8103.
E-mail: shirleen.roeder@yale.edu

TABLE 1

Yeast strains

Strain	Genotype
BR4316	*his4-260 leu2::CUP1,arg4-8 iTHR1 iURA3 iNAT iLEU2 MATa iADE2* / *HIS4 leu2::CUP1,arg4-8 iHYG MATα* *TRP1::sgs1-Δ795 ade2-1 ura3-1* / *trp1-289 ade2-1 ura3-1*
BR4310	Same as BR4316, but homozygous *sgs1Δ-KAN*
BR4633	*leu2::CUP1,arg4-8 iTHR1 iura3-1 MATα iADE2 trp1-289* / *leu2::CUP1,arg4-8 iura3-stu iNAT iLEU2 MATa trp1-289* *ade2-1 ura3-1* / *ade2-1 ura3-1*
BR4337	*HIS4 leu2-27 MATα iADE2 TRP1::sgs1-Δ795 ade2-1 ura3-1* / *his4-260 LEU2::sgs1-Δ795 MATa trp1-289 ade2-1 ura3-1* *URA3 arg4-nsp thr1-4* / *arg4-bgl THR1*
BR4474	Same as BR4337, but homozygous *sgs1Δ-KAN*
BR4357	Same as BR4337, but homozygous *spo11-HA-HIS6*
BR4475	Same as BR4337, but homozygous *spo11-HA-HIS6* and *sgs1Δ-KAN*

centromere-proximal CO, occurs during meiotic prophase. The exact mechanism underlying this type of segregation has been difficult to decipher due to the inability to analyze all four products of a single meiosis.

Meiotic recombination events are distributed nonrandomly throughout the genome. Genetic interference ensures that COs are placed at some distance from each other. Some regions of the genome undergo higher frequencies of recombination than others (GERTON *et al.* 2000). In general, the regions around centromeres are cold spots for meiotic recombination. This repression of recombination is dependent on a functional centromere and affects both COs and noncrossovers (NCOs) (LAMBIE and ROEDER 1986, 1988). The deficit in recombination near centromeres is due, at least in part, to a reduction in formation of the double-strand breaks that serve to initiate meiotic recombination (ZENVIRTH *et al.* 1992; BAUDAT and NICOLAS 1997).

SGS1 is the sole budding-yeast homolog of the RecQ family of helicases (GANGLOFF *et al.* 1994; WATT *et al.* 1995; MIYAJIMA *et al.* 2000). *sgs1* mutants form inviable spores (60–80% spore viability), apparently because they undergo missegregation leading to disomy (WATT *et al.* 1995). The random pattern of spore inviability observed in tetrads from *sgs1* tetrads is inconsistent with a deficiency of COs. In fact, *sgs1* mutants have been shown to undergo a 30–40% increase in meiotic crossing over compared to wild type (ROCKMILL *et al.* 2003). Consistent with an increase in crossing over, cytological studies indicate that meiotic nuclei from *sgs1* contain an increased number of synapsis inititation complexes (SICs) (ROCKMILL *et al.* 2003), which are believed to mark the sites of COs (AGARWAL and ROEDER 2000; FUNG *et al.* 2004). Further evidence that Sgs1 plays an antagonistic role in meiotic crossing over comes from the observation

that *sgs1* mutants suppress the CO defect in certain meiotic mutants (JESSOP *et al.* 2006).

We find that the major event leading to spore death in wild-type budding yeast is aneuploidy resulting from precocious separation of sister chromatids (PSSC) at meiosis I. The predominant products are disomes containing nonsister chromatids, and these are often associated with crossing over near the centromere. In addition, we show that the *sgs1* mutant undergoes the same type of chromosome missegregation events as wild type, and we provide evidence that the decrease in spore viability in *sgs1* is due to the increase in crossing over.

MATERIALS AND METHODS

Strains: Yeast strains are listed in Table 1. Diploids used to detect spores disomic for chromosome *III* (BR4316, BR4310, and BR4633) are homozygous for insertion of a single *CUP1* gene and an *arg4-8^{ts}* gene at the *LEU2* locus on chromosome *III* (SPECTOR and FOGEL 1992). Both genes are deleted from their normal locations on chromosome *VIII*. The *iTHR1*, *iHYG*, and *iNAT* markers ("*i*" indicates insertion at an ectopic location) were constructed by transforming with PCR products containing the markers and 55 nt of pericentromeric DNA to each side to place the markers ∼13 kb to the left of *CENIII* (*iTHR1* at CHR3 position 101398), ∼1.3 kb to the right of *CENIII* (*iHYG* at CHR3 position 115843), and ∼12 kb to the right of *CENIII* (*iNAT* at CHR3 position 125202). Oligonucleotide sequences are available upon request. The *iLEU2* marker 23 kb to the right of *CENIII* was made by transformation with pPGK1 (CLARKE and CARBON 1980). The *iURA3* and *iADE2* markers were made by transformation with plasmids pJC303-4 (CLARKE and CARBON 1983) and pCB432 (CHUA and ROEDER 1997), respectively.

The *URA3* heteroalleles present in BR4633 (Table 1, Figure 1C) were derived from Ura$^+$ haploid segregants of the wild-type diploid BR4316 (Table 1, Figure 1A). Replacing the *URA3* gene at the centromere with *ura3-1* and *ura3-stu* alleles was achieved by selection on 5-fluoro-orotic acid to select for uracil
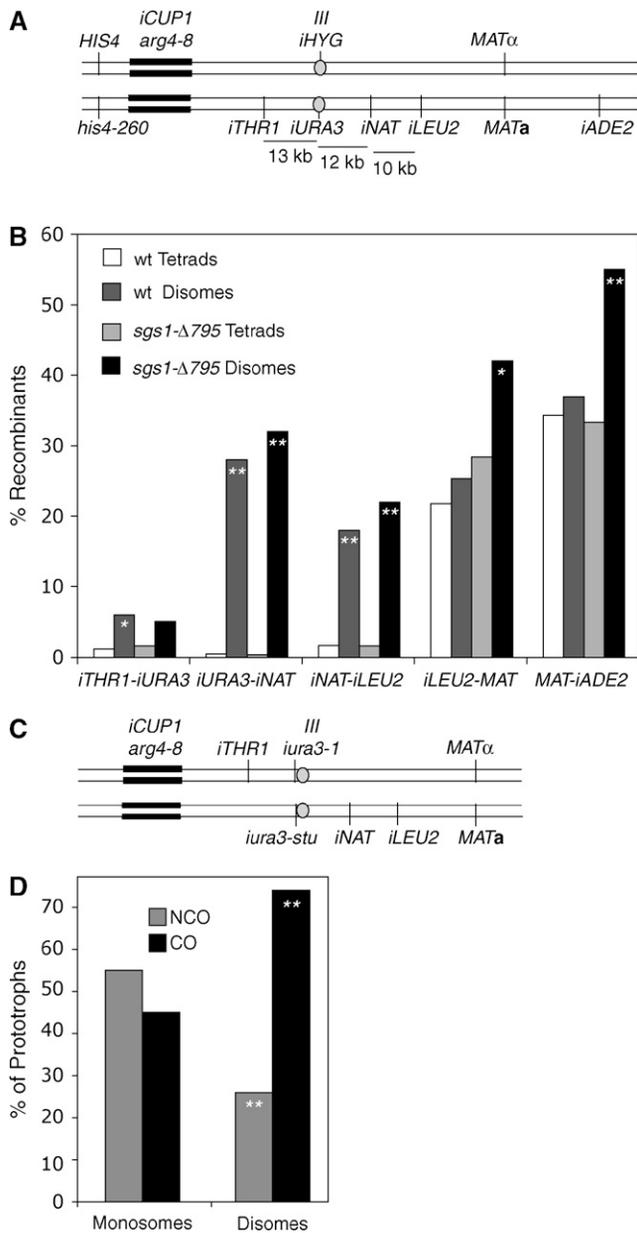
FIGURE 1.—Centromere-proximal crossovers are associated with disomy. (A) Configuration of markers in strains used for the detection and analysis of chromosome *III* disomy and associated recombination events. (B) Map distances in five intervals from four-spore viable tetrads (converted to random spore data) are compared to map distances found in disomic spores derived from wild type and *sgs1-Δ795* (MATERIALS AND METHODS). Intervals where map distances in disomic spores are significantly different from map distances in tetrads are indicated by asterisks (*, $P < 0.05$; **, $P \ll 0.001$). (C) Configuration of markers in the strain used to measure COs associated with gene conversion. (D) The frequency of crossing over associated with gene conversion is shown for monosomic Ura+ recombinants and for mating-competent Ura+ disomes. The results from monosomes and disomes are significantly different ($P < 0.0001$ using chi-square analysis).

auxotrophs (BOEKE *et al.* 1984) after transformation with PCR fragments containing these alleles. The *ura3-1* allele was amplified from genomic DNA; the *ura3-stu* allele was amplified from pAZ2a (ROCKMILL and ROEDER 1990).

Strains shown in Figure 3A (BR4337, BR4357, BR4474, and BR4475) are diploids in which both haploid parents are isogenic to BR1919-8B (ROCKMILL and ROEDER 1998). Diploids are heterozygous for *HIS4* on chromosome *III* and *THR1* on chromosome *VIII* (constructed by transformation with the corresponding wild-type genes). The *iADE2* allele distal to *MAT* was constructed by transformation with pCB432 (CHUA and ROEDER 1997). A wild-type copy of *LEU2* and a copy of the *sgs1-Δ795* truncation allele were inserted at the *LEU2* locus by transformation with pRL1 (MULLEN *et al.* 2000). The wild-type *TRP1* gene and another copy of the *sgs1-Δ795* allele were introduced by transformation with p303 (ROCKMILL *et al.* 2003). A wild-type copy of *URA3* was introduced upstream of the *ARG4* locus by transformation with pMJ177 (BORDE *et al.* 1999). The *sgs1-Δ795* strains are homozygous for a complete deletion of the *SGS1* open reading frame and carry two insertions of the *sgs1-Δ795* allele, one at *LEU2* and one at *TRP1* (ROCKMILL *et al.* 2003). The wild-type strains are identical except that they carry two wild-type copies of *SGS1* at the *SGS1* locus. The *spo11-HA* strains were made by transformation with pSK54 (KEE and KEENEY 2002). *ZIP3-GFP* was introduced into haploid segregants that were uracil auxotrophs by transformation with pSA219 (AGARWAL and ROEDER 2000).

Genetic analysis: Map distances were calculated from four-spore viable tetrads and significance (standard error) was calculated using the website: www.groik.com/stahl/. E. Hoffmann and R. Borts supplied a calculator for *G*-test analysis of spore viability. Comparison of map distances between disomes and tetrads was done by converting tetrad data into random spore data. Thus, map distance was calculated as the number of nonparental ditype (NPD) tetrads plus half the number of tetratype (TT) tetrads divided by the total number of tetrads.

Disomy was measured directly in 1300 tetrads from wild type (BR4316) and 1500 tetrads from an *sgs1-Δ795* strain (BR4310). The starting strains are diploids homozygous for a dosage-effect assay that allows chromosome *III* disomes to be detected (WHITTAKER *et al.* 1989). Meiotic segregants that are monosomic for chromosome *III* are copper sensitive (Cu^s) and arginine auxotrophic (Arg−), whereas meiotic segregants that are disomic for chromosome *III* are copper resistant (Cu^R) and arginine prototrophic (Arg+). Three-spore viable tetrads containing one disomic spore were scored for the segregation of a centromere marker, *TRP1*, to determine whether the disomic spore had a viable sister. If the sister was viable, then the disomic spore and one other viable spore had the same configuration for *TRP1* (*i.e.*, both were Trp+ or Trp−). In addition, disomes were scored for the presence of sister or nonsister chromatids of chromosome *III* by scoring the centromere markers *iURA3* and *iHYG*. Those that contained both markers were scored as having nonsister chromatids.

Hyg^R Ura+ spores were selected from a pool of purified spores (ROCKMILL *et al.* 1991) obtained from wild-type (BR4316) and *sgs1-Δ795* (BR4310) diploids. Those testing positive for disomy (*i.e.*, Arg+ and Cu^R) were scored for homozygosis of recessive markers on chromosome *III* to assess crossing over. The frequencies of Thr−, Nat^s, and Leu− spores were doubled to account for the inability to detect the reciprocal products (*e.g.*, *LEU2/LEU2*).

Ura+ spores were selected from a sporulated wild-type diploid (BR4633) containing *URA3* heteroalleles adjacent to *CENIII*. Recombination between flanking markers was scored directly in 700 Ura+ prototrophs monosomic for chromosome *III* (*i.e.*, Cu^s and Arg−). Mating-competent Ura+ disomes (*i.e.*, Cu^R Arg+) were crossed to a tester strain, and tetrad analysis was carried out to determine the configuration of chromosome *III* markers. Nonmating disomes were analyzed by PCR to determine heterozygosity of the flanking markers. A set of PCR primers for each marker was used to detect alleles

with and/or without the insert (*iTHR* and *iNAT*). Mating-competent disomes represent 46% of total disomes.

Cytology: Meiotic cells were spread and stained as previously described (Rockmill *et al.* 2003). The number of Zip3 foci was quantitated in nuclei with fully synapsed chromosomes.

RESULTS AND DISCUSSION

Chromosome missegregation in wild type: Wild-type strains of *Saccharomyces cerevisiae* produce a low level of inviable spores. To assess the contribution of chromosome missegregation to spore inviability, tetrad analysis was performed using a strain in which meiotic products containing two copies of chromosome *III* (disomes) can be detected. The starting diploid strain (BR4316, Figure 1A) is homozygous for insertion of the *CUP1* gene and the *arg4-8[ts]* gene at the *LEU2* locus on chromosome *III*. This strain is sensitive to copper and unable to grow in the absence of arginine at 30°. However, a disomic strain carrying two copies of chromosome *III* in an otherwise haploid genome is resistant to copper and able to make its own arginine at 30°. Thus, colonies derived from disomic spores can be identified by replica plating to medium containing copper and lacking arginine. The assay is not robust enough to allow disomes to be recovered quantitatively by plating spores on selective medium.

Of 1300 tetrads dissected, 1.5% contained at least one spore disomic for chromosome *III*. All tetrads containing one or more disomic spores also contained <4 viable spores. To determine whether the observed rate of chromosome missegregation can account for the spore inviability seen in wild type, the predicted level of spore viability was calculated on the basis of the assumption that the frequency of missegregation found for chromosome *III* is similar for the other 15 chromosome pairs. In 22% ($1 - (0.985)^{16}$) of meioses, a chromosome is expected to missegregate, rendering at least one meiotic product inviable. Since 1 meiosis results in 4 spores (a tetrad), at least 22 of 400 spores (100 meioses × 4 spores) would be inviable due to aneuploidy, predicting 94.5% spore viability (378/400). The spore viability observed is 91%. Thus, most spore inviability in wild type can be accounted for by aberrant chromosome segregation, if all chromosomes behave like chromosome *III*.

Missegregation occurs via PSSC: The path of chromosome segregation leading to disomy can be inferred from the analysis of three-spore viable tetrads harboring one disomic spore because all four chromatids are recovered. The segregation of a centromere marker on another chromosome (*TRP1* near *CENIV*) allows the identification of sister spores. In the majority (88%) of three-spore-viable, disome-containing tetrads, the disomic spore has a viable sister spore (Table 2). Furthermore, inspection of the centromere markers on the disomic chromosomes (*i.e.*, *iHYG* and *iURA3*, Figure 1A) reveals that most (72%) of the disomes contain nonsister chromosomes (*i.e.*, are Hyg⁺ Ura⁺) (Table 2). These observations suggest that for the majority of aber-

TABLE 2

Analysis of disomes recovered from tetrads

Genotype[a]	Viable sister[b]	Dead sister[b]	Hyg[R] Ura⁺ (MI)[c]	Hyg[R] or Ura⁺ (MII)[c]
Wild type	8	1	13	5
sgs1-Δ795	9	1	49	9

[a] Strains analyzed were BR4316 and BR4310.

[b] Tetrads in which three spores were viable and one spore was disomic for chromosome *III* were analyzed to determine whether the sister spore to the disome was viable.

[c] Disomes recovered from one-, two- and three-spore viable tetrads were scored for containing one or both centromere markers, representing the products of MI and MII missegregation events, respectively.

rant meioses, at least one chromosome pair underwent PSSC at meiosis I (Figure 2A).

COs near centromeres are enriched in disomes: To analyze a large sample of aneuploid meiotic products, disomic spores were selected from a wild-type diploid (Figure 1A). Disomes were recovered by selecting for both centromere *III* markers (*iURA3* and *iHYG*) from purified spores. These disomes contain two nonsister chromatids, corresponding to the predominant class of disomes recovered by tetrad analysis. COs in five intervals on chromosome *III* were scored in these disomes and compared to CO frequencies from four-spore viable tetrads (Tables 3 and 4). Disomes were scored for homozygosis of recessive markers on chromosome *III*, and this frequency was doubled to account for the inability to detect homozygosis of dominant alleles (*e.g.*, both *LEU2/LEU2* and *LEU2/leu2* spores are phenotypically Leu⁺).

The results in Table 4 and Figure 1B demonstrate a strong, but not absolute, correlation between centromere-proximal recombination events and the occurrence of disomes. For example, among four-spore-viable tetrads (chromosomes properly segregated), the rate of crossing over in the 13-kb *iURA3–iNAT* interval is 0.5%, but it is 28% in disomes, corresponding to a 56-fold increase. This increase falls off to 10-fold in the adjacent *iNAT–iLEU2* interval and 5-fold in the *iTHR1–iURA3* interval. Two centromere-distal intervals (*iLEU2–MAT* and *MAT–iADE2*) did not display increased COs in disomes. Thus, crossing over in the centromere-proximal region is strongly associated with PSSC. It should be noted, however, that a substantial number of disomes (almost half) do not appear to have undergone a centromere-proximal CO. These disomes may be due to COs that we cannot detect, or they may be unrelated to recombination.

COs, as opposed to NCOs, are preferentially associated with disome formation: Our data suggest that COs near the centromere interfere with chromosome segregation. However, an alternative possibility is that chromosomes that are prone to missegregate are also
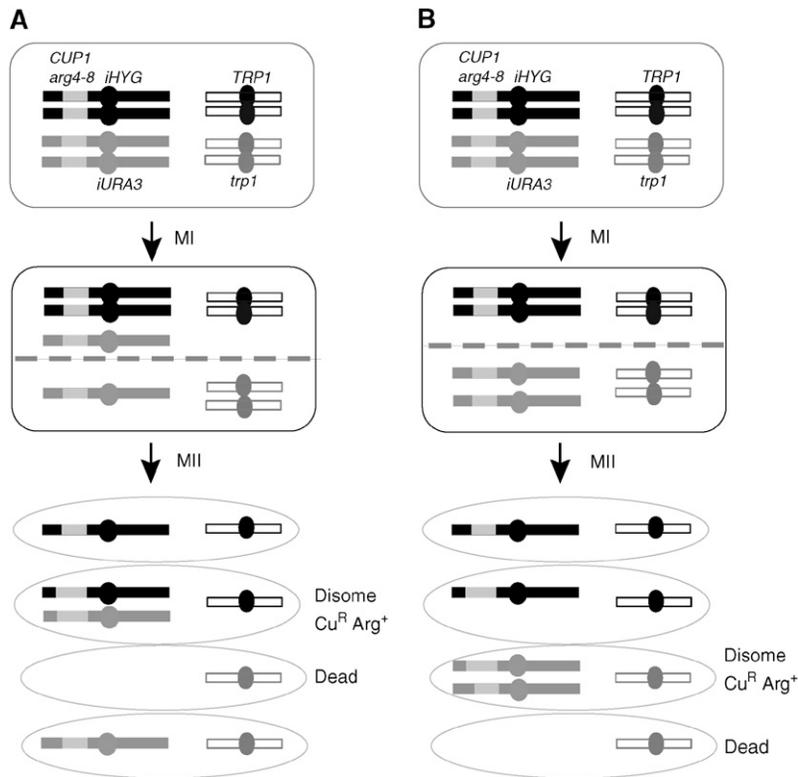
FIGURE 2.—Precocious separation of sister chromatids. (A) PSSC at meiosis I. Shown are two pairs of homologous chromosomes, with each chromosome consisting of two sister chromatids. The larger chromosomes carry the markers used to detect disomes (*arg4-8*, *CUP1*, bars with light shading). Circles represent centromeres. At meiosis I, the pair of small chromosomes segregates properly, while one of the large chromosomes undergoes PSSC. At meiosis II, intact pairs of sister chromatids undergo proper sister segregation, but single chromatids segregate randomly. This results in a tetrad in which one spore is disomic, containing nonsister chromatids, and this spore has a viable sister spore. Two spores are euploid, and one is inviable due to aneuploidy. (B) PSSC at meiosis II. Chromosomes segregate properly at meiosis I. At meiosis II, both sister chromatids segregate to the same pole, forming a disomic spore carrying sister chromatids and an inviable sister spore.

more likely to undergo recombination. For example, an altered chromatin structure near the centromere might predispose the chromosome to both events. In this scenario, PSSC should be associated with recombination events in general (including both COs and NCOs), and the relative frequencies of COs *vs.* NCOs should be the same in both monosomic and disomic spore populations. If, however, COs are preferentially associated with PSSC, then the relative frequency of COs to NCOs should be increased in disomes compared to monosomes.

To distinguish between these possibilities, a diploid was constructed in which mutant *URA3* genes are inserted immediately adjacent to *CENIII* (Figure 1C). Most uracil prototrophs (Ura$^+$) result from gene conversion events within *URA3*, which may or may not be accompanied by reciprocal exchange between flanking markers. Two different methods were used to measure crossing over in Ura$^+$ disomes. Disomes that were

mating competent were crossed to a tester strain, and the configuration of *iTHR1* and *iNAT* markers was determined through the analysis of meiotic progeny (Table 5). Disomes that were nonmaters were analyzed by PCR to score for heterozygosity or homozygosity at *iTHR1* and *iNAT*. By PCR analysis, it is not possible to distinguish disomes containing two recombinant chromatids (class B) from nonrecombinants (class A). These classes can be distinguished by tetrad analysis; thus, mating-competent disomes provide a more complete measure of CO frequency. The frequency of disomes carrying a single recombinant chromatid (classes D and E) is the same in mating-competent disomes as in nonmaters ($P = 0.32$ for class D; $P = 0.41$ for class E; $P = 1.0$ for classes D and E combined), arguing that mating-competent disomes are representative of the population at large. Thus, only mating-competent disomes are considered in the following discussion.

TABLE 3

Tetrad analysis of strains carrying centromere markers

Genotype[a]	iTHR1–iURA3 PD:NPD:TT[b]	cM[c]	iURA3–iNAT PD:NPD:TT[b]	cM[c]	iNAT–iLEU2 PD:NPD:TT[b]	cM[c]	iLEU2–MAT PD:NPD:TT[b]	cM[c]	MAT–iADE2 PD:NPD:TT[b]	cM[c]
Wild type	240:0:6	1.2	909:0:9	0.5	880:0:32	1.8	519:4:387	22.6	343:7:500	31.9
sgs1-Δ795	202:0:7	1.7	605:0:5	0.4	741:0:24	1.6	220:14:242	34.2[d]	220:22:218	38.0

[a] Strains analyzed were BR4316 and BR4310.
[b] PD, parental ditype; NPD, nonparental ditype; TT, tetratype.
[c] Map distances were calculated from four-spore viable tetrads using Perkins' formula (PERKINS 1949).
[d] Map distance is significantly different between wild type and mutant.

TABLE 4

Crossing over in Hyg[R] Ura[+] MI disomes

Genotype[a]	iTHR1–iURA3[b] (% CO[c])	iURA3–iNAT[d] (% CO[c])	iNAT–iLEU2[e] (% CO[c])	Total disomes	% CO
Wild type	9 (6)	39 (28)	26 (18)	282	52
sgs1-Δ795	3 (5)	20 (32)	14 (22)	126	59

[a] Strains analyzed were BR4316 and BR4310.

[b] Disomes that are auxotrophic for threonine represent COs in the iTHR1–iURA3 interval.

[c] To calculate CO frequency, the frequency of recombinants detected was multiplied by two to account for the inability to detect disomes homozygous for dominant alleles.

[d] Disomes that are sensitive to Clonat represent COs in the iURA3–iNAT interval.

[e] Disomes that are auxotrophic for leucine and resistant to Clonat (or sensitive to Clonat and leucine prototrophic) represent COs in the iNAT–iLEU2 interval.

Among Ura[+] spores monosomic for chromosome III, 45% had a CO in the CENIII region between iTHR1 and iNAT (Figure 1C). In contrast, among Ura[+] spores that were disomic, 74% had undergone a CO in the iTHR1-iNAT region (Table 5; Figure 1C), suggesting that COs (as opposed to NCOs) are more often associated with missegregation.

Among Ura[+] disomes, 36% sustained a CO in the iNAT–iLEU interval just to the right of the iTHR1–iNAT interval spanning the centromere (Figure 1C). In total, 88% of disomes had at least one CO in the overall iTHR1–iLEU2 region, supporting the notion that centromere-proximal COs predispose chromosomes to undergo PSSC. In addition, 21% of the disomes sustained two COs in the iTHR1–iLEU2 region. The occurrence of these rare double COs in disomes raises the possibility that multiple recombination events, perhaps even at some distance from the centromere, have an adverse effect on chromosome segregation.

sgs1 mutants display increased COs and increased PSSC: The sgs1-Δ795 mutant is a deletion of the C-terminal 795 amino acids, which includes the helicase domain (MULLEN et al. 2000). The sgs1-Δ795 strains used here carry a deletion of the SGS1 gene at the SGS1 locus; one copy of the sgs1-Δ795 gene is inserted at LEU2 and another at TRP1. The isogenic wild-type strains contain both sgs1-Δ795 inserts, but the wild-type SGS1 gene is present at its normal location on both homologs.

To determine if the spore inviability in sgs1-Δ795 strains is due to PSSC, tetrad analysis was performed on an sgs1-Δ795 strain (BR4310) carrying the chromosome III markers diagrammed in Figure 1A. The frequency of disomes was 3.5%, more than twice the frequency in wild type. This frequency is probably an underestimate, since disomes are particularly unstable in the sgs1-Δ795 background (data not shown), likely leading to a failure to recover a portion of the chromosome III disomes. This rate of disomy formation predicts 86% spore viability, if all chromosomes are equally affected. Spore viability in the sgs1-Δ795 strain is 77%, suggesting that at least half of the spore death occurs via PSSC. In three-spore viable tetrads containing a disome, most disomes (90%) have a

viable sister spore and most disomes (84%) contain non-sister chromatids (Table 2), similar to wild type.

To determine whether disomes from sgs1-Δ795 are enriched for crossing over, disomes were selected from an sgs1-Δ795 diploid (BR4310) and examined for CO frequencies on chromosome III. Similar to wild type, disomes from sgs1-Δ795 are enriched for recombination in the centromeric region (Table 4, Figure 1B). Unlike wild type, the centromere-distal intervals also display significant increases in COs in disomic spores. This observation raises the possibility that missegregating chromosomes from sgs1-Δ795 have sustained disproportionate numbers of COs.

In both wild type and sgs1-Δ795, COs in the region to the right of CENIII have a stronger effect on PSSC than those occurring to the left. Interestingly, the region to the right of CENIII is enriched for cohesins and the centromere-binding protein, Sgo1, suggesting that the functional centromere extends beyond the 117-bp CENIII sequence sufficient for centromere activity on a replicating plasmid (BLAT and KLECKNER 1999; KIBURZ et al. 2005). This correspondence between the location of heightened cohesin deposition and the region in which COs predispose to missegregation supports the notion that COs perturb kinetochore function in meiosis, perhaps through disruption of sister-chromatid cohesion.

Reducing COs in sgs1 improves spore viability: As noted above, crossing over is increased in sgs1-Δ795 strains; it is possible that some of the excess COs occur near centromeres and thus promote PSSC. If the excess COs in sgs1-Δ795 are deleterious, then reducing crossing over should improve spore viability. Spo11 is the endonuclease responsible for catalyzing the double-strand breaks (DSBs) that initiate meiotic recombination (KEENEY et al. 1997). In strains homozygous for the tagged SPO11 allele, spo11-HA, the formation of meiotic double-strand breaks is reduced to ~70% of the wild-type level (MARTINI et al. 2006, our unpublished data).

Combining the spo11-HA allele with sgs1-Δ795 reduces crossing over to approximately wild-type levels (Table 6, Figure 3B). Accordingly, the number of Zip3

TABLE 5

Crossing over in disomic Ura$^+$ gene convertants

Class	Genotype of disome	Maters[a] (%)	Nonmaters[b] (%)
A	Het. *iTHR1* Het. *iNAT* (parental configuration)[c]	14 (24)	NA
B	Het. *iTHR1* Het. *iNAT* (nonparental configuration)[d]	20 (34)	NA
C	Het. *iTHR1* Het. *iNAT* (configuration unknown)[e]	NA	38 (59)
D	Hom. *iTHR1* Het. *iNAT*[f]	14 (24)	10 (15)
E	Het. *iTHR1* Hom. *iNAT*[f]	9 (16)	15 (23)
F	Hom. *iTHR1* Hom. *iNAT*[g]	1 (2)	2 (3)
	% CO frequency	74[h]	>38[i] (72[j])

Disomic Ura$^+$ gene convertants selected from spores of BR4633 were analyzed for crossing over in the *iTHR1-iNAT* interval. Het., heterozygous; Hom., homozygous; NA, not applicable.

[a] A total of 58 mating-competent disomes were scored by tetrad analysis.

[b] A total of 65 nonmating disomes were analyzed by PCR.

[c] The two chromatids retained the parental configuration of markers. One carried *iTHR1*, but not *iNAT*; the other carried *iNAT*, but not *iTHR1*.

[d] Both chromatids displayed a recombinant configuration of markers. One carried both *iTHR1* and *iNAT*; the other chromatid carried neither marker.

[e] Classes A and B cannot be distinguished by PCR analysis.

[f] These classes result from crossing over in the *iTHR1–iNAT* interval.

[g] This class represents MII disomes carrying sister chromatids.

[h] CO frequency was calculated as the sum of classes B, D, and E divided by the sum of all classes and multiplied by 100.

[i] CO frequency represents the minimum frequency; it is the sum of classes D and E divided by the sum of all classes and multiplied by 100.

[j] CO frequency was calculated on the basis of the assumption that class C contains disomes with the nonparental configuration of markers (*i.e.*, like class C) and that the frequency of these disomes relative to those with the parental configuration (*i.e.*, like class B) is the same as it is among mating-competent disomes. Thus, the CO frequency was calculated as the sum of classes D and E plus 0.59 × class C divided by the sum of all classes and multiplied by 100.

foci is restored to the wild-type level (Figure 3, C–E). Spore viability in the double mutant is significantly increased compared to the *sgs1-Δ795* single mutant (Figure 3F), implying that much of the spore death in *sgs1-Δ795* mutants is due to excessive meiotic recombination. Taken together with the observations that most chromosome missegregation is due to PSSC and centromere-proximal COs are enriched in disomes, these results suggest that many of the CO events that are deleterious occur near centromeres.

These results are consistent with the proposed function for Sgs1 and other RecQ homologs as CO suppressors (KAROW *et al.* 2000; IRA *et al.* 2003; WU and HICKSON 2003; HU *et al.* 2005; JESSOP *et al.* 2006). It is reasonable for vegetative or somatic cells to employ a mechanism to prevent reciprocal recombination events, since loss of heterozygosity can be deleterious and predispose to cancer (LASKO and CAVENEE 1991). However, it is less clear why such an activity would be useful during meiosis, where crossing over is essential. One reason, suggested by our results, is that too much crossing over leads to missegregation due to COs near centromeres. In addition, some fraction of recombination events may be aberrant; for example, they might involve interactions between ectopic sequences or they may generate recombination intermediates with unligatable ends. It would be beneficial for the cell to be able to prevent or reverse such interactions. It is possible that aberrant events account for the inability of *spo11-HA* to restore spore viability to fully wild-type levels in *sgs1-Δ795*.

Meiotic cells induce about three times as many recombination events as will become COs (FOGEL *et al.* 1979). The phenotype of *sgs1* mutants suggests that Sgs1 functions in wild type to reduce the number of COs to the appropriate level. When the number of DSBs is reduced by *spo11-HA* in an otherwise wild-type background, the level of COs is unchanged (MARTINI *et al.* 2006) (Figure 3C). This result suggests the existence of a mechanism to ensure that the wild-type level of COs is attained, despite a reduction in the total number of initiated events, a phenomenon referred to as "CO homeostasis." Perhaps Sgs1 and CO homeostasis are part of the same mechanism. In strains with a reduced level of DSBs, CO homeostasis may act by downregulating Sgs1 activity. This interaction could explain why the *sgs1-Δ795* mutation has little effect in a *spo11-HA* background.

Disomes containing sister chromatids: Although most of the disomes recovered from tetrad analysis are composed of nonsister chromatids, a significant number contain sister chromatids (Table 3). Disomes of this type are difficult to recover because they cannot be selected from random spores (they are not Hyg^R Ura$^+$). Nevertheless, information is available from the limited number of disomes detected during tetrad analysis. From wild-type and *sgs1* strains combined, a total of 14 disomes containing sister chromatids were recovered; 6 of these experienced a CO in the *CENIII* region. Thus, centromere-proximal COs are associated with both MI and MII disomes. Segregation could be determined for four tetrads in which three spores were viable. In one tetrad, the chromatids segregated to the same pole at meiosis II, as shown in Figure 2B. Interestingly, in the three remaining tetrads, both pairs of chromosomes *III* missegregated. One pair segregated to opposite poles at meiosis I as shown in Figure 2A, and the other pair segregated to the same pole at meiosis II as shown in Figure 2B. In all three cases, this resulted in a tetrad in which the disome containing sister chromatids had a viable sister spore.

Meiotic PSSC in other organisms: As noted in the Introduction, the major source of meiotic aneuploidy in

TABLE 6

Tetrad analysis of *spo11-HA* diploids

Genotype[a]	HIS4–LEU2 PD:NPD:TT[b]	cM[c] (fold[d])	LEU2–MAT PD:NPD:TT[b]	cM[c] (fold[d])	MAT–iADE PD:NPD:TT[b]	cM[c] (fold[d])	ARG4–THR1 PD:NPD:TT[b]	cM[c] (fold[d])
Wild type	218:7:123	23.7 (1.0)	180:5:165	27.9 (1.0)	123:12:228	41.3 (1.0)	298:0:37	5.5 (1.0)
sgs1-Δ795	275:19:187	31.3[e] (1.3)	193:19:266	39.7[e] (1.4)	179:34:309	49.1 (1.2)	428:0:60	6.1 (1.1)
spo11-HA	379:6:255	22.7 (1.0)	276:10:367	32.7 (1.2)	128:23:278	48.5 (1.2)	584:0:65	5.0 (0.9)
sgs1-Δ795 spo11-HA	392:1:160	15.0[e] (0.6)	318:11:224	26.2 (0.9)	251:24:324	39.1 (0.9)	515:0:61	5.3 (1.0)

[a] Strains analyzed were BR4337, BR4474, BR4357, and BR4475.
[b] PD, parental ditype; NPD, nonparental ditype; TT, tetratype.
[c] Map distances were calculated from four-spore viable tetrads using Perkin's formula (PERKINS 1949).
[d] The fold increase in map distance relative to wild type is indicated.
[e] Map distances are significantly different from those in wild type.

Drosophila and humans results from the failure to cross over, leading to nondisjunction at meiosis I (KOEHLER *et al.* 1996; ORR-WEAVER 1996; HASSOLD and HUNT 2001; LAMB *et al.* 2005). We have shown that this is not the case in yeast, since most meiotic aneuploidy results from PSSC and the missegregating chromatids undergo increased crossing over near the centromere and normal levels of crossing over elsewhere.

A second type of missegregation observed in flies and humans results in disomes containing sister chromatids

and is associated with recombination near the centromere (KOEHLER *et al.* 1996; LAMB *et al.* 1996). Two models have been proposed to account for these events (KOEHLER *et al.* 1996). The first model suggests that an entanglement of the bivalent resulting from retention of sister-chromatid cohesion prevents segregation at meiosis I. Both homologs go to the same pole at meiosis I and then undergo reductional segregation at meiosis II. This model predicts the formation of two viable meiotic products, both of which are disomes containing
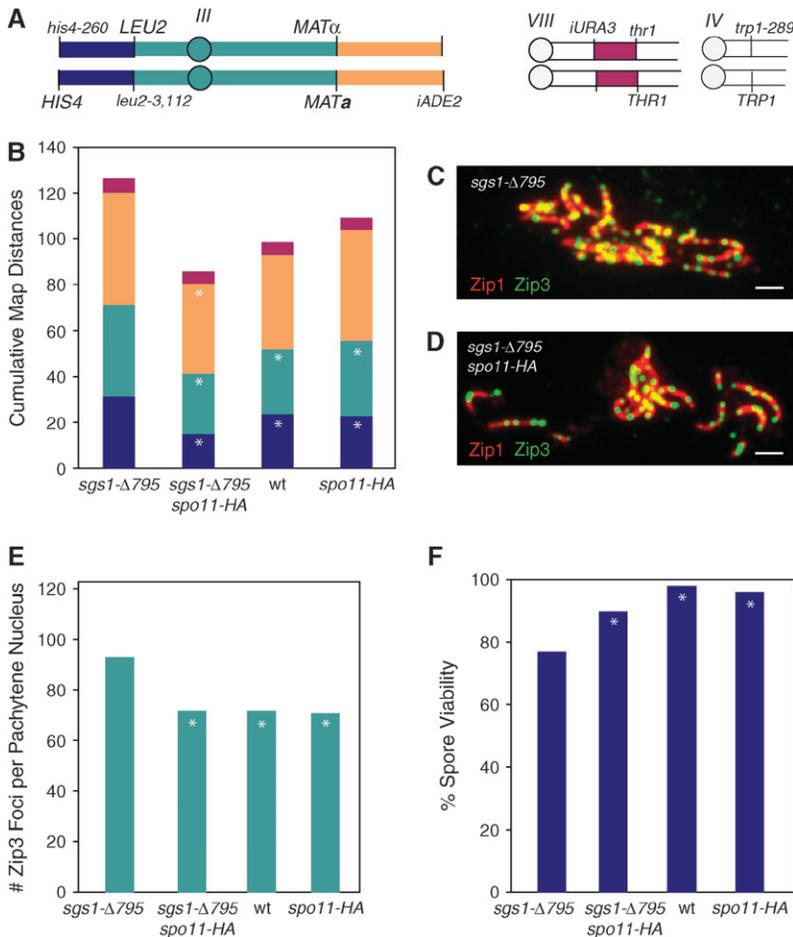
FIGURE 3.—*SPO11-HA* suppresses *sgs1-Δ795*. (A) Configuration of markers in strains used for analysis of crossing over in various mutant backgrounds. (B) COs were measured in four intervals in wild type (BR4316), *sgs1-Δ795* (BR4474), *spo11-HA* (BR4357), and *sgs1-Δ795 spo11-HA* (BR4475). Intervals are designated by the color utilized in A. Asterisks denote intervals in which map distances are significantly different from those in *sgs1* ($P < 0.05$). (C) Spread nucleus from *sgs1-Δ795* cell at pachytene stained for Zip3-GFP (green) and Zip1 (red) in pachytene. Yellow indicates regions of overlap. Bar, 1 μm. (D) Spread nucleus from *sgs1-Δ795 spo11-HA* stained as in C. Bar, 1 μm. (E) Zip3-GFP foci were quantified in pachytene-staged chromosome spreads. Asterisks denote strains in which the number of Zip3 foci is significantly different from that of *sgs1-Δ795* ($P < 0.05$ using the *t*-test). (F) Spore viability. Differences between strains were assessed using the big *G*-test with spore viability patterns (*i.e.*, four-, three-, two-, one-, and zero-spore viable, not shown). Asterisks denote strains in which spore viability is significantly different from that of *sgs1-Δ795*.

sister chromatids. A second model proposes that sister chromatids separate at anaphase I. Nevertheless, both sister chromatids usually continue to the same pole at meiosis I, and this is followed by random segregation at meiosis II. Occasionally, however, the chromatids segregate away from each other at meiosis I to generate disomic gametes containing nonsister chromatids.

The ability to do tetrad analysis in yeast allows us to distinguish between these different patterns of chromosome missegregation. We observed no examples of tetrads predicted by the first model (*i.e.,* two viable disomic spores containing sister chromatids), implying that this model does not apply to yeast. Yeast disomes predominantly contain nonsister chromatids, but a substantial number have sister chromatids. It is likely that the same mechanism underlies both events, since both types of disomes are associated with centromere-proximal COs.

It is attractive to assume that PSSC events associated with centromere-proximal COs occur by the same mechanism in yeast and animals. Our results support a model in which chromatid separation is initiated at meiosis I, but the chromatids can segregate away from each other either at meiosis I or at meiosis II (Figure 2). The difference between PSSC in animals and yeast may simply be the timing of chromatid separation. If chromatids do not fully separate until homologs have already begun to move away from each other at meiosis I, then the two sisters may continue their movement to the same pole. In this case, the resulting disomes would carry sister chromatids, as is most often the case in mammals and flies (Figure 2B). However, if the chromatids separate at or just prior to the metaphase I to anaphase I transition, then the sisters may segregate away from each other at the first division. In this case, the resulting disomes would carry nonsister chromatids, as is usually the case in yeast (Figure 2A).

Perhaps the difference in the timing of sister chromatid separation during PSSC events in yeast *vs.* animals reflects underlying differences in kinetochore structure. Whereas each kinetochore in budding yeast is attached to a single microtubule, the kinetochores of higher eukaryotes have multiple microtubules attached (Joglekar *et al.* 2006). Disrupting the monopolar orientation of a chromosome with a compound kinetochore might be more difficult than disrupting a single microtubule attachment.

COs may disrupt centromere structure: Our studies have revealed that COs are strongly associated with PSSC, suggesting that COs can lead to loss of the pericentric cohesion needed for proper alignment of homologs at metaphase I. Indeed, cytological observations of chiasmata reveal that sister-chromatid cohesion is disrupted at CO sites (*e.g.,* Eijpe *et al.* 2003; Parra *et al.* 2004; Kleckner 2006).

A recent model of centromere structure (Bloom *et al.* 2006) postulates the existence of an intramolecular loop resulting from the chromatid folding back on itself in the region of the centromere. The site of microtubule attachment is located at the tip of the loop, and the loop is stabilized by cohesins. The loop is believed to be dynamic, growing shorter or longer, as more or less of the chromatid is incorporated into the loop. The authors postulate that the dynamic nature of the loop plays a role in the tension-based checkpoint mechanism that ensures proper chromosome segregation. COs that occur close to the centromere might interfere with formation and/or expansion of the loop, thus predisposing the affected chromosome to missegregate.

The correlation between pericentric COs and PSSC found in yeast, as well as in Drosophila and mammals, suggests that limiting the number of COs near the centromere may be a strategy that organisms employ to safeguard against loss of centromeric cohesion.

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Communicating editor: M. Lichten