Recombination Can Partially Substitute for *SPO13* in Regulating Meiosis I in Budding Yeast

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ABSTRACT

Recombination and chromosome synapsis bring homologous chromosomes together, creating chiasmata that ensure accurate disjunction during reductional division. *SPO13* is a key gene required for meiosis I (MI) reductional segregation, but dispensable for recombination, in *Saccharomyces cerevisiae*. Absence of *SPO13* leads to single-division meiosis where reductional segregation is largely eliminated, but other meiotic events occur relatively normally. This phenotype allows haploids to produce viable meiotic products. Spo13p is thought to act by delaying nuclear division until sister centromeres/chromatids undergo proper cohesion for segregation to the same pole at MI. In the present study, a search for new *spo13*-like mutations that allow haploid meiosis recovered only new *spo13* alleles. Unexpectedly, an unusual reduced-expression allele (*spo13-23*) was recovered that behaves similarly to a null mutant in haploids but to a wild-type allele in diploids, dependent on the presence of recombining homologs rather than on a diploid genome. This finding demonstrates that in addition to promoting accurate homolog disjunction, recombination can also function to partially substitute for *SPO13* in promoting sister cohesion. Analysis of various recombination-defective mutants indicates that this contribution of recombination to reductional segregation requires full levels of crossing over. The implications of these results regarding *SPO13* function are discussed.

MEIOSIS is the process by which diploid organisms reduce their chromosome number by half to produce haploid gametes for sexual reproduction. It is a specialized type of cell division whose genetic control has many components in common with mitosis. The most striking difference between mitotic and meiotic nuclear division is the separation of homologs at meiosis I (MI) in a reductional division. Below, two sisters segregating to the same pole will be referred to as "reductional chromosome behavior" (irrespective of whether homologous chromosomes go to opposite poles). Three properties of meiotic chromosomes act to form a bivalent that will undergo proper reductional division (reviewed in Moore and Orr-Weaver 1998). First, meiotic recombination between nonsister chromatids creates chiasmata, forming physical links between homologs that direct accurate disjunction. Second, cohesion between sister chromatid arms is necessary to maintain the chiasmata, which persist until the onset of anaphase in MI. Third, sister centromere cohesion ensures that the chromatids of each homolog coorient and remain together during meiosis I and keeps them together until they segregate to opposite poles at meiosis II.

In organisms in which recombination is a normal part of meiosis, reciprocal exchange has been shown to be essential for proper separation of homologs since they segregate randomly at MI in recombination-defective (Rec$^-$) mutants (reviewed in Baker *et al.* 1976; Kupiec *et al.* 1997). An example of this occurs in *Saccharomyces cerevisiae spo11* mutants, which are defective in double-strand break formation (Keeney *et al.* 1997). Such mutants are Rec$^-$ and execute two meiotic segregation events, producing mature spore products that are almost always inviable due to aneuploidy resulting from disordered MI segregation (Klapholz and Esposito 1982). Sister cohesion prevents separation of homologs upon resolution of recombination intermediates even though the arm region distal to the crossover site is now covalently linked to the opposite homolog (reviewed in Bickel and Orr-Weaver 1996; Moore and Orr-Weaver 1998). Recombination and sister cohesion place the MI bivalent under tension due to opposing forces between chiasmata (keeping homologs together) and polar microtubules (pulling homologs apart), which balance one another, promoting stable attachment of the bivalent to the spindle (Nicklas 1967).

Subsequently, the differential release of sister chromatid cohesion at arms and centromeres directs chromosome segregation at both divisions (reviewed in Bickel and Orr-Weaver 1996; Moore and Orr-Weaver 1998). At metaphase of MI, sister centromeres are cohered and cooriented toward the same spindle pole. At the onset of anaphase I, dissolution of arm cohesion (distal to chiasmata) releases homolog connections, allowing segregation to opposite poles. Cohesion/coorientation of sister centromeres, however, remains until metaphase of meiosis II, at which point they become

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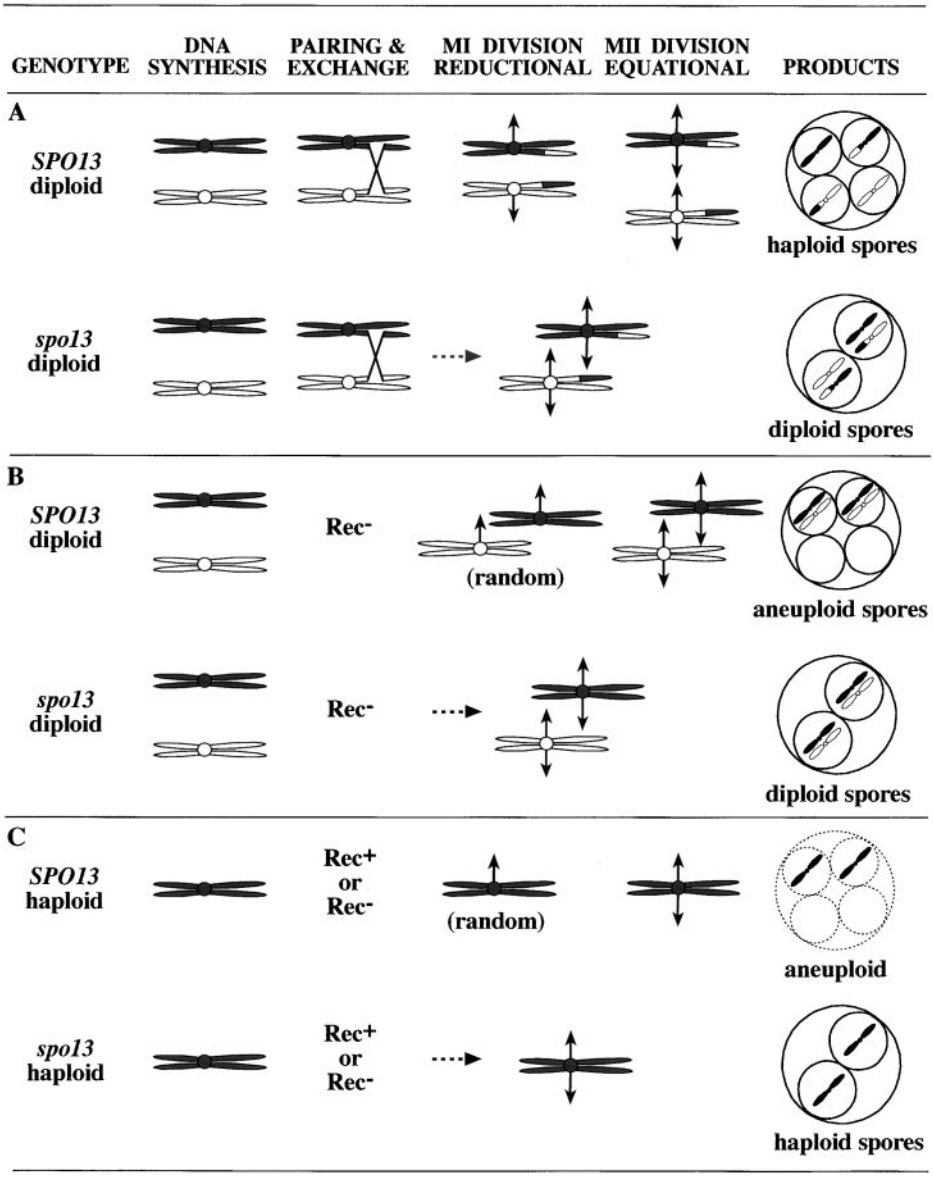

Figure 1.—Meiotic progression in wild-type and *spo13* meiosis. This figure shows the meiotic behavior of a single pair of homologous chromosomes (gray and white). (A) Wild-type diploids execute all meiotic landmarks and produce tetrads with haploid spores. *spo13* diploids undergo premeiotic DNA synthesis, pairing, and exchange. They then complete a single meiotic division. In many strains, most chromosomes undergo an equational (MII-like) division and produce dyads with diploid spores genetically similar to the starting diploid (except for the effects of recombination). (B) Recombination-defective *SPO13* diploids undergo random segregation at MI, producing tetrads with aneuploid, inviable spores. Since *spo13* mutants do not execute reductional segregation, such aneuploidy is eliminated in *spo13* Rec$^-$ strains, which can produce dyads with diploid, viable spores. (C) *MAT* heterozygous *SPO13* haploids also undergo random segregation at MI, producing univalent chromosomes in aneuploid tetranucleate cells that do not form mature spores. The absence of reductional division in *spo13* mutants allows haploids to complete a single meiotic division and produce dyads with haploid, viable spores.

bi-oriented and attach to microtubules from opposite poles of the spindle. At anaphase II, sister centromere cohesion dissolves, finally allowing equational segregation of sister chromatids.

The *SPO13* gene, which is dispensable for meiotic recombination, is also required for MI segregation. The *spo13-1* allele was identified along with *spo12-1* in a strain of *S. cerevisiae*, ATCC4117, known to undergo a single nuclear division during sporulation (Klapholz and Esposito 1980a). *spo13* mutants produce two-spored asci (dyads) with diploid spores resulting from a single meiotic division (Klapholz and Esposito 1980b). In many strain backgrounds, including W303 (used for this study), most chromosomes undergo equational division (Figure 1A). Reductional segregation is specifically defective while other events of meiosis are largely unaffected, including premeiotic DNA synthesis, recombination, equational segregation, and spore formation (Klapholz and Esposito 1980b). Interestingly, *spo13* mutants allow production of viable meiotic products even when there are no recombining homologous chromosomes (*e.g.*, Rec$^-$ diploids and haploids; Malone and Esposito 1981; Wagstaff *et al.* 1982). This occurs presumably because the single, largely equational division eliminates the lethal effects of random reductional segregation and resulting aneuploidy. The fact that sister chromatids segregate from one another during the single division implies that sister centromere cohesion is absent during the division. What then directs the accurate (rather than random) equational disjunction of sister chromatids in these mutants? By analogy to mitotic division, we presume that sister arm cohesion must occur normally in a *spo13* mutant and provide the proper tension for stable microtubule attachment and regular equational segregation (see Biggins and Murray 1999).

Three pieces of data, however, demonstrate that *SPO13* is not absolutely required for reductional division and that sister centromere cohesin assembly/stabilization can occur in the absence of *SPO13*. First, within the largely equational division seen in many *spo13* strains, individual chromosomes may behave aberrantly, where one member of a pair segregates equationally (sisters apart), while the other segregates reductionally (sisters together; Klapholz and Esposito 1980b). Second, in some strain backgrounds *spo13* diploids have been reported to undergo either a single, largely reductional meiotic division (Hollingsworth and Byers 1989), or a single mixed division in which individual chromosomes segregate reductionally or equationally (Hugerat and Simchen 1993). Significantly, in all of the above cases, when initiation of recombination is blocked, aberrant segregation is virtually eliminated and little or no reductional segregation is seen. Finally and most dramatically, the *spo13* deletion phenotype is partially suppressed and reductional division is restored when meiosis is slowed by sporulation either at low temperature or in the presence of hydroxyurea (McCarroll and Esposito 1994).

How does *SPO13* function? *SPO13* encodes a 291-amino acid protein with no striking homology to known proteins (Buckingham *et al.* 1990). Its transcription is repressed during vegetative growth and specifically induced (~70-fold) during meiosis with maximal levels at about the time of MI (Wang *et al.* 1987; Buckingham *et al.* 1990). We have proposed that *SPO13* specifically regulates sister centromere cohesion without affecting cohesion of sister arms. A clue as to how this might occur comes from the observation that mitotic overexpression of *SPO13* (*e.g.*, from a galactose-inducible promoter) causes a *CDC28*-dependent cell cycle arrest at the metaphase-to-anaphase transition (McCarroll and Esposito 1994). Meiotic overexpression of *SPO13* also inhibits progression of M phase during MI, but instead of arresting cells, it acts as a transient negative regulator, significantly delaying the first meiotic division compared to control cultures (McCarroll and Esposito 1994). These observations led to the model that *SPO13* promotes reductional segregation through a *CDC28*-dependent mechanism that delays the metaphase-to-anaphase transition at MI until chromosomes establish/stabilize sister centromere cohesion. As predicted by this model, other conditions that lead to similar delays (*e.g.*, low temperature, hydroxyurea treatment) can substitute for *SPO13* and promote reductional division (McCarroll and Esposito 1994).

Over the last several years, significant progress has been made in understanding the molecular basis of sister chromatid cohesion (reviewed in Biggins and Murray 1999). A number of mitotic cohesins, as well as some meiosis-specific homologs, including the Rec8 proteins of budding and fission yeast, have been identified and studied (Molnar *et al.* 1995; Klein *et al.* 1999; Parisi *et al.* 1999). Rec8, which is required for centromere cohesion, is initially localized along the length of pachytene chromosome cores and later becomes restricted to sister centromeres at the time of the MI division, persisting until the onset of anaphase II (Klein *et al.* 1999). The recent finding that centromere localization of Rec8 during meiotic divisions is dependent on *SPO13* in baker's yeast (Klein *et al.* 1999) lends support to the view that *SPO13* regulates centromere cohesion. It is not yet known whether this occurs by allowing time for factor(s) to stabilize/protect Rec8 in this region or by a more direct mechanism (see discussion).

The aim of this study was to recover new *spo13*-like mutants in order to identify other components of the *SPO13*-dependent centromere cohesion pathway. During the course of this analysis, a reduced-expression allele, *spo13-23*, was isolated. Using this allele and two others with similar phenotypes, we found that *SPO13* function and recombination have partially redundant roles in sister centromere cohesion during MI reductional segregation. These studies and their implications for *SPO13* function are discussed below.

MATERIALS AND METHODS

Strains: The genotypes of *S. cerevisiae* strains are listed in Table 1. All strains constructed in this study were derived by genomic integration of markers into the isogenic haploids W303-1A and W303-1B (R. Rothstein) and confirmed by Southern blot analysis. Haploids with various markers were crossed and tetrad analysis was used to recover appropriate segregants.

Gene duplications used to monitor intrachromosomal recombination (*trp1-1::URA3::trp1-3′Δ* and *can1-100::ADE2::CAN1^s*) were made by integration of pRS19 containing *URA3* and *trp1-3′Δ* (Strich *et al.* 1986) at *trp1-1* and pADECAN (Fritze *et al.* 1997) at *can1-100*, respectively. Deletion and other mutant alleles of *SPO13* were constructed as follows. The *spo13-Δ4* complete deletion allele was made by two-step gene replacement (Rothstein 1991) using the plasmid pCM103 (this laboratory). The *spo13::hisG* allele was made by one-step gene replacement of the wild-type *SPO13* allele (Rothstein 1983) with the *spo13 5′::hisG::URA3::hisG::spo13 3′* cassette from pNKY58 (gift of N. Kleckner) followed by selection for loopout of the *URA3* marker (Alani *et al.* 1987). Various mutant alleles, *spo13-23*, *spo13-9*, or *spo13-10*, were introduced by two-step gene replacement of the wild-type gene using the plasmids pBE917, pBE918, and pBE919, respectively. Various recombination-defective mutant alleles were constructed as follows. A *spo11* deletion allele constructed in this laboratory (C. Atcheson), *spo11-Δ3 yhl021c::HIS3*, includes a disruption of the gene immediately upstream, *YHL021C*, with *HIS3*. *yhl021c* mutants have no detectable phenotype in mitosis or meiosis. *red1::LEU2* and *mek1::LEU2* disruption alleles were constructed by one-step gene replacement using the plasmids pB72 (Rockmill and Roeder 1988) and pB118 (Rockmill and Roeder 1991), respectively (gifts of G. S. Roeder). *msh4::KanMX* and *msh5::KanMX* deletions were constructed by replacement of the corresponding wild-type genes with a KanMX6 cassette using short flanking PCR-generated homology (Wach 1996). Heterozygous centromere-proximal markers were introduced into the W303 strain to monitor meiotic reductional and equational segregation. A *TRP1* (chromo-

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype		Source
W303a	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*		R. Rothstein
W303α	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*		R. Rothstein
REE2999[a]	*MAT*α *can1-100:ADE2:CAN1 trp1-1:URA3:trp1*Δ		This study
REE1660	*MAT***a** *ade2 can1 his7-2 leu1-12 lys2-1 met13-d spo13-1 trp5-d ura3*		This laboratory
	W303-derived near isogenic haploid pairs[a,b]		
REE3457	*MAT***a** *LEU2*		This study
REE3441	*MAT*α *TRP1 URA3*		This study
REE3463	*MAT***a** *LEU2 TRP1 spo11-*Δ*3(yhl021c::HIS3)*		This study
REE3464	*MAT*α *URA3 spo11-*Δ*3(yhl021c::HIS3)*		This study
REE3462	*MAT***a** *LEU2 URA3 spo13-*Δ*4*		This study
REE3461	*MAT*α *TRP1 spo13-*Δ*4*		This study
REE3459	*MAT***a** *LEU2 spo11-*Δ*3(yhl021c::HIS3) spol13-*Δ*4*		This study
REE3403	*MAT*α *TRP1 URA3 spo11-*Δ*3(yhl021c::HIS3) spo13-*Δ*4*		This study
REE3468	*MAT***a** *URA3 spo13-23*		This study
REE3469	*MAT*α *LEU2 TRP1 spo13-23*		This study
REE3470	*MAT***a** *TRP1 URA3 spo11-*Δ*3(yhl021c::HIS3) spo13-23*		This study
REE3471	*MAT*α *LEU2 spo11-*Δ*3(yhl021c::HIS3) spo13-23*		This study
	W303-derived isogenic haploid pairs[a,b]		
*MAT***a**	*MAT*α		
REE3237	REE3236	*spo13-*Δ*4*	This study
REE3386	REE3387	*spo13-23*	This study
REE3400	REE3401	*spo11-*Δ*3(yhl021c::HIS3)*	This study
REE3290	REE3289	*spo11-*Δ*3(yhl021c::HIS3) spo13-*Δ*4*	This study
REE3399	REE3398	*spo11-*Δ*3(yhl021c::HIS3) spo13-23*	This study
REE3448	REE3449	*spo13-9*	This study
REE3474	REE3475	*spo11-*Δ*3(yhl021c::HIS3) spo13-9*	This study
REE3535	REE3536	*spo13-10*	This study
REE3586	REE3587	*LEU2 URA3 spo11-*Δ*3(yhl021c::HIS3) spo13-10*	This study
REE3482	REE3483	*mek1::LEU2*	This study
REE3480	REE3481	*mek1::LEU2 spo13-*Δ*4*	This study
REE3484	REE3485	*mek1::LEU2 spo13-23*	This study
REE3488	REE3489	*red1::LEU2*	This study
REE3486	REE3487	*red1::LEU2 spo13-*Δ*4*	This study
REE3490	REE3491	*red1::LEU2 spo13-23*	This study
REE3621	REE3622	*msh4::KanMX*	This study
REE3623	REE3624	*msh4::KanMX spo13-*Δ*4*	This study
REE3625	REE3626	*msh4::KanMX spo13-23*	This study
REE3627	REE3628	*msh5::KanMX*	This study
REE3629	REE3630	*msh5::KanMX spo13-*Δ*4*	This study
REE3631	REE3632	*msh5::KanMX spo13-23*	This study

[a] All of these strains have the same genotype as W303, except as indicated.
[b] *MAT***a** and *MAT*α haploid pairs were crossed to make diploids for sporulation experiments.

some IV) haploid was recovered from a mitotic loopout recombination event at the *trp1-1::URA3::trp1-3′*Δ marker. *LEU2* (chromosome III) and *URA3* (chromosome V) strains were made by transformation of mutant haploid strains with restriction fragments containing wild-type alleles of these genes.

Plasmids: Plasmids used in this study (Table 2) were manipulated using standard methods (Maniatis *et al.* 1989). High-copy (2 μm) *SPO13* and *spo13-23* plasmids (pBE983, pBE984) were made by cloning the *Bam*HI-*Xmn*I fragments from p(SPO13)8 and pBE902 (see below) into the *Bam*HI-*Sma*I sites of pRS426. The plasmids p9-45-2 (*spo13-9*) and p10-46-2 (*spo13-10*) were isolated in a hydroxylamine mutagenesis of the plasmid pCM103 (R. McCarroll and R. E. Esposito, unpublished results). *Bam*HI-*Hin*dIII fragments from these plasmids were cloned into the *Bam*HI-*Hin*dIII sites of YIp5, creating the allele replacement plasmids pBE918 and pBE919, respectively.

Yeast methods, growth, and sporulation: Standard methods used for yeast growth (Adams *et al.* 1997), sporulation (Klapholz and Esposito 1982; Klapholz *et al.* 1985), and transformation (Chen *et al.* 1992; Johnston 1994) have been described. Percentage of asci on SPIII plates (2% potassium acetate, 0.1% dextrose, 0.25% yeast extract, 75 mg/liter of required amino acids, 1.8% Bacto agar) was scored after 5 days of incubation at either 23° (for haploids) or at 30° (for diploids), unless otherwise noted. Approximately 300 cells for each of three independent clones were counted. Spore viability was determined from at least three independent dissections of 20 tetrads or 40 dyads each.

Isolation of suppressors of reductional segregation: The

TABLE 2

Plasmids

Plasmid	Plasmid type	Description	Source
pBE272	YCp	*LEU2* vector with *MAT***a**	S. Gottlieb
pBE273	YCp	*LEU2* vector with *MAT*α	S. Gottlieb
pRS19	YIp	pUC7 with *URA3* and *trp1* deleted from nt 426–827	R. Strich
pADECAN	YIp	pUC19 containing *ADE2* and *CAN1*	C. Fritze
p(SPO13)8	YCp	YCp50 containing *SPO13*	L. Buckingham
pBE902	YCp	YCp50 containing *spo13-23*	This study
pBE983	YEp	pRS426 containing *SPO13*	This study
pBE984	YEp	pRS426 containing *spo13-23*	This study
pBE524	YCp	pRS313 containing *spo13-9*	R. McCarroll
pBE525	YCp	pRS313 containing *spo13-10*	R. McCarroll
pNKY58	YIp	pBR332 with *spo13*::*hisG* disruption cassette	N. Kleckner
pCM109	YIp	pRS306 two-step *spo13*Δ plasmid	R. McCarroll
pBE917	YIp	YIp5 containing *spo13-23*	This study
pBE918	YIp	YIp5 containing *spo13-9*	This study
pBE919	YIp	YIp5 containing *spo13-10*	This study
pB72	YIp	Used to create *red1*::*LEU2* allele	G. S. Roeder
pB118	YIp	Used to create *mek1*::*LEU2* allele	G. S. Roeder

haploid strain REE2999 bearing the plasmid pBE272 (*MAT***a** *LEU2*) was mutagenized with ethyl methanesulfonate (EMS) to a level of 30% survival (Guthrie and Fink 1991). Survivors were plated on synthetic growth media lacking leucine to select for the plasmid. The resulting colonies were replica plated to SPIII and incubated for 5 days at 30°. To select for completion of recombination and segregation, cells were replica plated to canavanine-containing media. The Can^r survivors result from either loopout or gene conversion between *CAN1* alleles, followed by segregation of the recombined (*can1*^{r}) allele from the parental (*CAN1*^{s}) allele. Surviving Can^r clones were patched to master plates and retested for ability to undergo meiotic recombination using the *trp1-1*::*URA3*::*trp1*-Δ marker and selecting for Trp$^+$ recombinant survivors on media lacking tryptophan. Isolates surviving both the initial selection and rescreening were considered candidate mutant suppressors of reductional segregation.

Recovery and localization of the *spo13-23* mutation: The EMS-induced *spo13-23* allele was recovered from chromosome VIII by gap repair (Rothstein 1991). The gapped vector was prepared from a *Bst*EII/*Xba*I digest of p(SPO13)8, gel purified and transformed into the *spo13-23* mutant haploid. Plasmids from several independent isolates were recovered and transformed into *spo13*Δ strains to test their sporulation phenotype. One plasmid had the *spo13-23* phenotype and was named p(spo13-23)8. The *spo13* allele on this plasmid was sequenced and found to contain more than four differences from the published sequence (Saccharomyces genomic resources, Stanford University). Restriction fragment swapping between p(SPO13)8 and p(spo13-23) more precisely localized the *spo13-23* mutation to a region upstream of the open reading frame. This region had only two single-base changes from the published *SPO13* sequence. These were introduced individually by PCR site-directed mutagenesis (Vallejo *et al.* 1995) into a wild-type *SPO13* allele. The *spo13-23* phenotype was found to be due to a G to A change at position −129 upstream of the AUG initiating codon. This allele was cloned into YIp5 to construct the allele replacement plasmid pBE917. All experiments described here (except those in Figure 3) were done using this reintroduced *spo13-23* allele.

S1 nuclease protection assays: Total RNA was isolated from growth and sporulation culture samples by the glass bead/phenol protocol (Ausubel *et al.* 1991). S1 nuclease protection analysis was performed using 20 μg of total RNA for each hybridization as previously described (Elder *et al.* 1983). *SPO13* (Surosky and Esposito 1992) and *DED1* (Tevzadze *et al.* 2000) RNA probes were synthesized in *in vitro* transcription reactions. *DED1* mRNA was used as a loading control since its levels are constant during meiosis (C. Atcheson, G. Tevzadze and R. E. Esposito, unpublished observations). *DED1* is an essential gene thought to be required for mRNA translation initiation (Struhl 1985; Chuang *et al.* 1997). Both *SPO13* and *DED1* probes were used at concentrations shown to give a linear relationship between the signal and the amount of RNA used in the hybridization (data not shown). Quantitation of *SPO13* and *DED1* (control) signals in S1 gels was done with a Molecular Dynamics PhosphorImager using ImageQuant software.

Genetic determination of aberrant segregation: Aberrant segregation, in which one spore segregates equationally and the other segregates reductionally (sisters together), was examined using three heterozygous centromere-proximal markers, *TRP1*, *URA3*, and *LEU2*. In this study, Rec$^+$ *spo13*-Δ*4* and double mutant *spo11 spo13*-Δ*4* and *spo11 spo13-23* strains were all observed to produce dyads containing apparent reductional segregation (one spore + and the other spore −) for one of three markers tested. These dyads were analyzed further to distinguish whether the +:− segregation resulted from either a reductional (+/+; −/−) or aberrant (+/+/−; +) division. The wild-type diploid spore from each dyad tested (either +/+ or +/+/−) was transformed with either a *SPO13* plasmid [p(SPO13)8] for *spo13*-Δ*4* spores, or a *SPO11* plasmid [pBE979] for *spo13-23* spores. These were then sporulated, tetrads were dissected, and the resulting spore clones were scored for segregation of the marker in question. If the dyad diploid spore clone was +/+ for the marker, only + haploid spores will be recovered. If the dyad diploid spore clone was +/+/− for the marker, then both + and − haploid spores will be found based on trisomic segregation (Klapholz and Esposito 1980a). All dyads tested were found to result from aberrant segregation. Chromosome segregation was also examined in *msh4 spo13*-Δ*4* and *msh4 spo13-23* dyad asci, using the same three centromere-proximal markers. In this case, the level of aberrant *vs.* reductional segregation was deter-

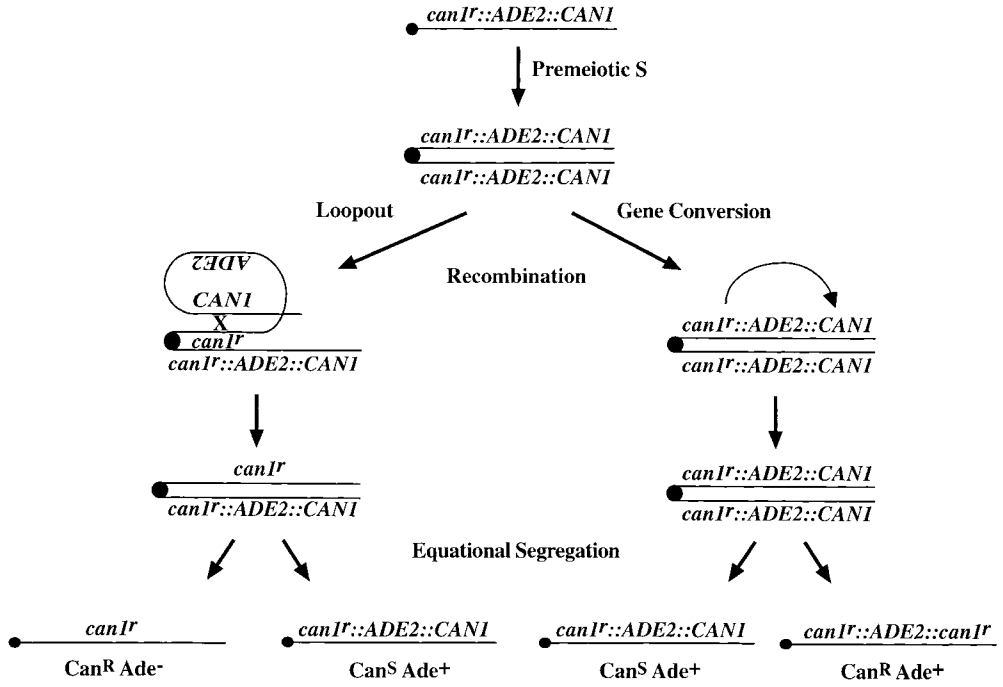

Figure 2.—Selection for recombination and equational segregation. An intrachromosomal recombination marker (*can1*^{r}*::ADE2::CAN1*^{s}) is used to select for both recombination and equational segregation as shown. Following premeiotic DNA synthesis, homologous recombination between the *CAN1* alleles results in either a loopout excision or a gene conversion event, leaving either *can1*^{r} or *CAN1*^{s} allele(s) on that chromatid. If the former event is followed by equational segregation of the parental chromatid (*can1*^{r}*::ADE2::CAN1*^{s}) from the recombined chromatid (*can1*^{r} or *can1*^{r}*::ADE2::can1*^{r}), one of the spores in the resulting dyad will be canavanine resistant (Can^r). The appearance of Can^r spores thus reflects completion of both recombination and equational segregation.

mined for chromosome III on the basis of segregation of the codominant mating type locus.

RESULTS

spo13* mutants appear to be unique in allowing haploids to produce viable meiotic products:** To identify genes that act with *SPO13* to control the meiosis I division, novel *spo13*-like mutants were sought by selecting for alleles that suppress reductional division and allow haploid meiosis. Although wild-type haploids expressing both *MATa** and *MAT*α can enter meiosis, execute two meiotic divisions, and form tetranucleate cells, they do not form viable products. Since haploids contain only a single homolog for each chromosome, reductional chromosome behavior (sisters together segregation) leads to extensive aneuploidy and failure of packaging of mature spores (Wagstaff *et al.* 1982). To select for haploid meiosis, a *MAT*α haploid containing a plasmid bearing *MAT***a** was mutagenized with EMS. The strain contained two gene duplications, *can1*^{r}*::ADE2::CAN1*^{s} and *trp1-1::URA3::trp1-3′*Δ. The first was used to simultaneously select for intrachromosomal recombination and equational segregation after incubation on sporulation medium by recovery of Can^r clones (Figure 2; materials and methods). The second duplication provided a rapid assay to rescreen candidates for meiotic recombination proficiency by recovery of Trp$^+$ prototrophs.

Among 160,000 survivors of mutagenesis, 10 putative mutants that produce mature dyads with viable spores during meiosis were identified by this procedure (Figure 3, top). All mutant isolates were tested for *spo13* complementation in two ways. First, complementation was assayed in diploid meiosis by crossing to a *spo13-1* null haploid and scoring for the percentage of tetrad and dyad asci (Figure 3, bottom). Second, complementation was examined in haploid meiosis by transformation with a wild-type *SPO13* plasmid (data not shown). These tests demonstrated that 9 out of the 10 mutants contain *spo13* alleles and have phenotypes virtually identical to the *spo13* null. The remaining mutant also proved to contain an allele of *SPO13*, but exhibited unusual behavior as described in the next section. Given these data, the probability of another nonessential gene with the same phenotype was calculated at $\sim 1 \times 10^{-3}$. This calculation assumes that two equally mutable genes with the same mutant phenotype are present in the genome; if this were the case, then the probability of isolating only one of them 10 times is $(0.5)^n$ where $n = 10$. On the basis of the large number of independent *spo13* alleles recovered and the failure to isolate mutations in other genes, we conclude that mutations in *spo13* are likely to be unique in their ability to suppress reductional segregation and permit haploid meiosis.

An unusual reduced-expression allele behaves similarly to a *spo13* null in haploid meiosis and a *SPO13* wild type in diploid meiosis: A novel mutant (#23) was recovered that produces dyad asci in haploid meiosis similar to a *spo13*Δ null mutant (Figure 3, top). However, when crossed to a *spo13-1* null mutant, the resulting diploid produces tetrads similar to a wild-type strain (Figure 3, bottom). Segregation analysis confirmed that this mutation (#23) resides in the *SPO13* gene. Among 20 tetrads, all segregated 0:4 for *spo13*, assayed by their ability to produce dyads with viable spores in haploid meiosis. The novel allele was designated *spo13-23*.

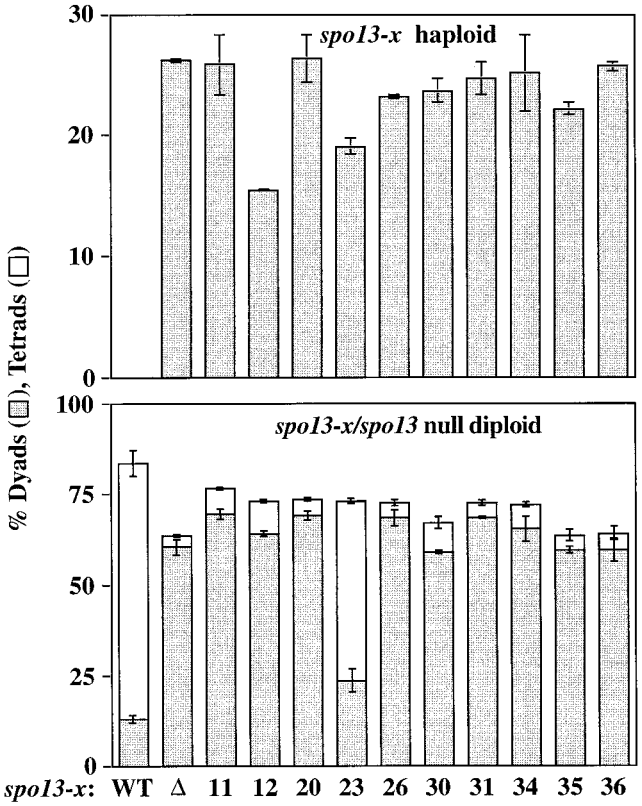

Figure 3.—Sporulation phenotypes of new alleles of *spo13*. The top shows dyad production (solid bars) in haploid mutants capable of sporulation. The bottom shows dyad (solid bars) and tetrad (open bars) production in these mutants when crossed to a *spo13-1* null mutant, REE1660.

To understand the molecular nature of *spo13-23*, the mutation was recovered from the genome, localized, and sequenced. The *spo13-23* phenotype was found to be due to a G to A change in the promoter at position −129 upstream of the translational start codon (Figure 4A). S1 nuclease protection analysis demonstrated that *spo13-23* mRNA is repressed normally during mitosis and is induced at about the same time as the wild-type mRNA (Figure 4B; Steber and Esposito 1995). However, peak expression reaches only one-half of wild-type levels, indicating that *spo13-23* is a reduced-expression allele (Figure 4B). Since the mutation is in a region known to be needed for full levels of transcriptional activation in meiosis (L. Buckingham and R. E. Esposito, unpublished observations), we postulate that it disrupts function of an element needed for full levels of meiotic activation. Studies are underway to define this element.

If the only defect of the *spo13-23* allele is reduced expression of a wild-type protein, then increasing its gene dosage should restore a wild-type phenotype. This was found to be the case. Comparison of the ability of *spo13-23* and *SPO13* single-copy (CEN) plasmids and similar high-copy (2 μm) plasmids to complement the *spo13*Δ phenotype in Rec$^+$ diploids, Rec$^-$ diploids, and haploids showed that single-copy *spo13-23* complements only ∼65% as well as *SPO13*. In contrast, high-copy *spo13-23* and *SPO13* complement to about the same extent (data not shown).

Reductional segregation in *spo13-23* mutants is dependent on recombination and not ploidy: To determine if the differential behavior of this allele in diploids and haploids is related to diploidy or the presence of recombining chromosomes, the sporulation phenotype of *spo13-23* was examined in Rec$^+$ and Rec$^-$ diploids. If diploidy *per se* is required for reductional division independent of recombination, then tetrads should be produced in both Rec$^+$ and Rec$^-$ *spo13-23* diploids. Alternatively, if recombination is the critical factor, then *spo13-23* strains should produce tetrads in Rec$^+$ diploids and dyads in Rec$^-$ diploids. In these studies, the Rec$^-$ phenotype was conferred by the presence of a *spo11* mutation. *SPO11* encodes the enzyme required to catalyze double-strand break formation (Keeney *et al.* 1997). Mutants in this gene are completely defective in meiotic recombination and although they execute both divisions, the spores produced are inviable due to aneuploidy resulting from random segregation at MI (Klapholz and Esposito 1982). The type of meiotic chromosome division (reductional *vs.* equational) was examined in these experiments using heterozygous, centromere-proximal markers on three different chromosomes. In addition, spore viability was measured to gauge meiotic division accuracy since spores produced from inaccurate divisions have reduced viability due to aneuploidy.

As expected, all strains containing the wild-type *SPO13* gene (Rec$^+$ or Rec$^-$) execute two meiotic divisions, while those containing a *spo13* null allele undergo a single meiotic division (Figure 5). *SPO13* wild-type Rec$^+$ diploids produce tetrads with high spore viability. Those lacking recombining homologs (Rec$^-$ diploids and Rec$^+$ haploids) complete two meiotic divisions, but fail to form viable spores due to catastrophic reductional segregation. All *spo13* deletion strains (Rec$^+$ or Rec$^-$ diploids and Rec$^+$ haploids) produce dyads with two viable spores (see Figure 5). These data are consistent with previously published results (Malone and Esposito 1981; Klapholz and Esposito 1982; Wagstaff *et al.* 1982; Klapholz *et al.* 1985).

In contrast, the number of divisions in *spo13-23* mutants varies, specifically dependent on the presence of recombining homologs rather than diploidy. As described earlier, *spo13-23* haploids behave like the *spo13* null (Figures 3 and 5). Rec$^+$ *spo13-23* diploids execute both reductional and equational divisions, producing tetrads with near wild-type spore viability (93 *vs.* 96%). On the other hand, in the absence of recombination, *spo13-23* diploids lose the ability to form tetrads and instead behave like *spo13* null strains, bypassing reductional segregation and executing a single equational segregation, producing dyads. Spore viability in the dyads derived from *spo13-23* Rec$^-$ diploids is moderately

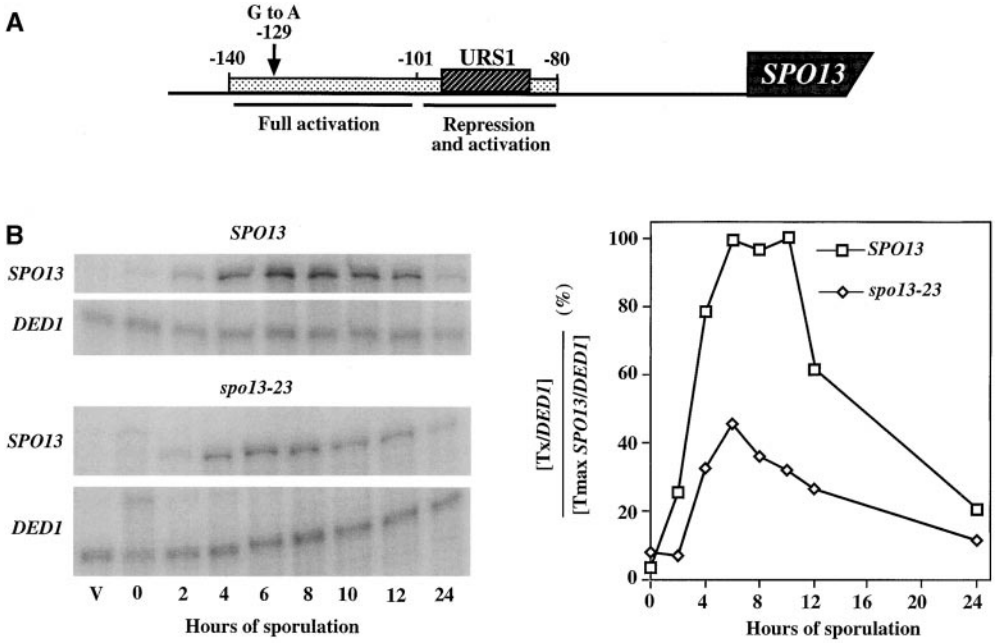

Figure 4.—Location and expression of *spo13-23*. (A) The region from −80 to −140 upstream of the *SPO13* open reading frame is necessary and sufficient for regulation of *SPO13* transcription. The fragment from −80 to −101 contains URS1 and directs both mitotic repression and meiotic activation. The region from −101 to −140 is also necessary for full levels of meiotic activation. The *spo13-23* mutation is a G to A change located at position −129. (B) S1 nuclease protection analysis of *SPO13* mRNA levels in wild-type (W303a × W303α) and *spo13-23* (REE3386 × REE3387) diploids in vegetative cells (V) and during a meiotic time course experiment (left). The *DED1* transcript is used as a loading control. *SPO13*/*DED1* mRNA levels are quantitated and expressed as a percentage of the wild-type maximum levels (*SPO13*+, squares; *spo13-23*, diamonds; right). Samples collected from a parallel *spo13*Δ (REE3237 × REE3236) meiotic time course experiment were used as a negative control (data not shown).

reduced compared to *spo13* null Rec⁻ diploids (54 *vs.* 89%), suggesting that the equational division in *spo13-23* is slightly less accurate than in the null. Spore viability of haploid *spo13-23* and *SPO13* wild-type dyads is nearly identical (53 *vs.* 54%). Since the number of divisions in *spo13-23* diploids depends on recombination and since Rec⁻ diploids and haploids behave similarly, we conclude that the presence of recombination can substitute for reduced *SPO13* function. To test the unlikely possibility that *spo13-23* mRNA accumulates to a higher level in Rec⁺ *vs.* Rec⁻ strains, we performed S1 analysis of *spo13-23* mRNA in *SPO11* and *spo11* diploids. Since the mRNA accumulation was the same in both strains, these results confirmed that the difference in phenotype is due to a difference in recombination and not due to an indirect effect on *SPO13* transcription (data not shown).

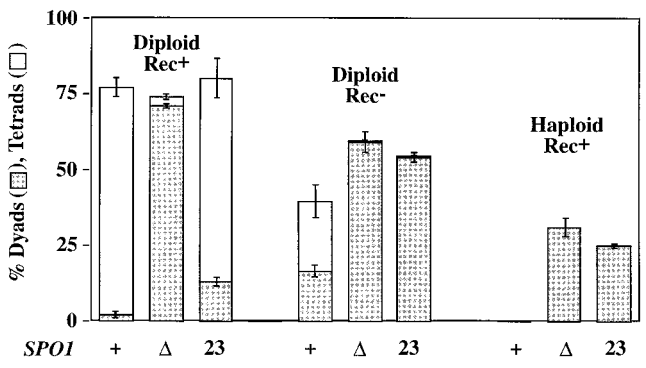

Figure 5.—Sporulation phenotypes of *spo13-23*. Graph showing production of dyads (solid bars) and tetrads (open bars) in Rec⁺ diploids, Rec⁻ (*spo11*) diploids, and Rec⁺ haploids in *SPO13*, *spo13-Δ4*, and *spo13-23* strains listed in Table 1. Spore viability in these strains was as follows. Rec⁺ diploids: *SPO13* (96%), *spo13-Δ4* (40%), *spo13-23* (93%); Rec⁻ (*spo11*) diploids: *SPO13* (>2%), *spo13-Δ4* (89%), *spo13-23* (54%); haploids: *SPO13* (ND), *spo13-Δ4* (53%), *spo13-23* (54%).

In previous studies of the *spo13* phenotype, individual chromosomes were often seen to exhibit aberrant behavior, in which one homolog segregates equationally and the other reductionally (sisters together), resulting in one spore containing three chromatids and the other spore containing one chromatid for that chromosome (Klapholz and Esposito 1980b). Aberrant segregation was similarly observed in some dyads in this study. For the three heterozygous centromere-linked markers used in this analysis, dyads were seen in which two of the markers segregated $2^+:0^-$ as expected for equational segregation (both spores +/−), and the other segregated $1^+:1^-$ consistent with either reductional (one spore +/+ and the other −/−) or aberrant (one spore +/+/− and the other −) segregation. Genetic analysis of wild-type spore clones from representative dyads was performed to distinguish among these latter possibilities (see materials and methods). For each dyad tested in all strains, aberrant segregation was found to be the cause of the $1^+:1^-$ dyad. Levels of aberrant segregation for the *spo13* null were consistent with previous observations (∼11% in *SPO11 spo13*Δ mutants and ∼1% in *spo11 spo13*Δ; Klapholz and Esposito 1980b). Aberrant segregation in *spo13-23* Rec⁻ diploids (2%) is similar to that seen in the Rec⁻ *spo13*Δ null. It should be noted that these are minimum estimates of aberrant segregation since the frequency of this event can be measured only in viable spores.

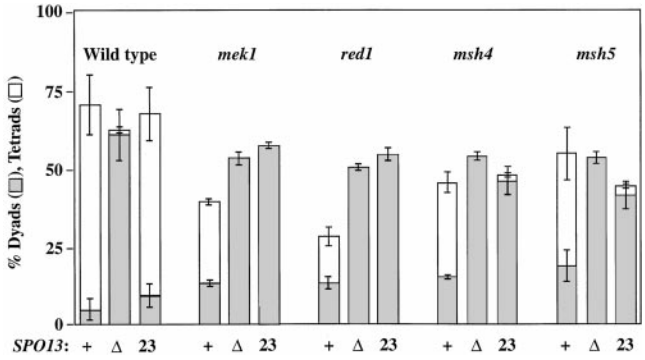

Figure 6.—Sporulation of *spo13-23* when recombination and synapsis are reduced. Graph showing production of dyads (solid bars) and tetrads (open bars) in *red1*, *mek1*, *msh4*, and *msh5* diploids in *SPO13*, *spo13-Δ4*, and *spo13-23* strains listed in Table 1. Spore viability in these Rec$^-$ diploid strains was as follows: *red1*: *SPO13* (7%), *spo13-Δ4* (83%), *spo13-23* (80%); *mek1*: *SPO13* (8%), *spo13-Δ4* (85%), *spo13-23* (74%); *msh4*: *SPO13* (83%), *spo13-Δ4* (60%), *spo13-23* (54%); *msh5*: *SPO13* (39%), *spo13-Δ4* (85%), *spo13-23* (66%).

Full levels of chromosome synapsis and crossing over are needed for two-division meiosis in *spo13-23* mutants: The analysis described above, demonstrating that the two-division meiosis in *spo13-23* mutants is dependent on recombination, utilized the severe Rec$^-$ mutant *spo11Δ*, which does not form meiotic double-strand breaks or undergo meiotic recombination (Klapholz and Esposito 1982; Klapholz *et al.* 1985; Borts *et al.* 1986; Giroux *et al.* 1989; Bergerat *et al.* 1997; Keeney *et al.* 1997). To determine whether full levels of recombination and synapsis are necessary to promote reductional segregation in *spo13-23* strains, a number of other Rec$^-$ mutants that affect later stages of the recombination process were examined, including *red1*, *mek1*, *msh4*, and *msh5*.

The *red1* and *mek1* mutants have either no or defective synaptonemal complex (SC), reduced levels of meiotic recombination, and produce mature tetrads with low spore viability, resulting from missegregation during reductional division (Rockmill and Roeder 1990, 1991; Leem and Ogawa 1992; Nag *et al.* 1995; Mao-Draayer *et al.* 1996; Schwacha and Kleckner 1997; Xu *et al.* 1997; de los Santos and Hollingsworth 1999). Like *spo11* double mutants, both *red1 spo13-23* and *mek1 spo13-23* execute single-division meiosis and produce only dyads (Figure 6). Spore viability of these dyads (80 and 74%, respectively) is similar to *spo13* null double mutants (83 and 85%, respectively), suggesting that their single equational division is reasonably accurate. These results indicate that the partial recombination activity, including formation and resolution of double-strand breaks, formation of interhomolog crossovers, and partial assembly of tripartite SC structures (particularly in *mek1* mutants), is not sufficient to promote reductional division when *SPO13* levels are reduced (Figure 6).

In contrast to the other Rec$^-$ mutants examined, the *msh4* and *msh5* mutants have near normal SCs and gene conversion. They exhibit defects at later stages of meiosis in resolution of recombination intermediates, resulting in at least a twofold reduction in crossing over in a number of intervals and a moderate level of MI nondisjunction and reduced spore viability (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Pochart *et al.* 1997). Significantly, both *msh4 spo13-23* and *msh5 spo13-23* double mutant diploids produce predominantly dyads (Figure 6) with spore viabilities of 60 and 66%, respectively. These values are only moderately lower than those observed for the *msh4* and *msh5 spo13-Δ4* double mutants, 83 and 85%, respectively. The fact that dyads rather than tetrads are produced, even in the presence of normal gene conversion and synaptonemal complex, suggests that near wild-type crossing over between homologs is necessary to compensate for reduced *SPO13* function. Why is this the case? One possibility is that even a modest reduction in crossing over eliminates sisters together reductional segregation and that this leads to equational segregation and dyad production when Spo13 is limiting. Alternatively, reductional chromosome behavior of some, but not all, chromosomes may still occur, but not at a level sufficient to effect the switch from dyads to tetrads.

To distinguish between these possibilities, the frequency of either aberrant or reductional behavior was further examined in *msh4* mutants using centromere-proximal markers on three independent chromosomes (III, IV, and V), as previously described. Strikingly, the level of aberrant or reductional behavior in *msh4 spo13-23* mutants is substantially higher than in *msh4 spo13-Δ4* (55 *vs.* 11% per chromosome per meiosis; Table 3). Reductional division of chromosome III was also monitored using the *MAT* locus (detected by dyads with one *MAT***a**/**a** spore and one *MATα*/*α* spore). In this case, *msh4 spo13-23* dyads exhibit 35% reductional division for chromosome III compared to <2% for *msh4 spo13-Δ4* (Table 3). These results demonstrate that despite the relatively high frequency of reductional behavior occurring in *msh4 spo13-23* mutants, this level is apparently not sufficient to trigger the dyad to tetrad switch. This raises two interesting questions of how many chromosomes are required to segregate reductionally to trigger two meiotic divisions in *spo13-23* mutants and how is this monitored by the cell (see discussion).

Other *spo13* mutations located in the coding region result in a phenotype similar to *spo13-23*: To determine whether other alleles have the same phenotype as *spo13-23*, two additional mutations known to partially complement a *spo13Δ* meiosis, *spo13-9* and *spo13-10* (R. McCarroll, unpublished results), were integrated into the genomic locus and examined in Rec$^+$ and Rec$^-$ diploids and haploids during sporulation. Both *spo13-9* and *spo13-10* have the same general phenotype as *spo13-23*, although *spo13-9* appears to be a slightly weaker (less

TABLE 3

Chromosome segregation in *msh4 spo13* mutants

Strains	Dyads dissected[a]	Dyads with two viable spores	Reductional/aberrant dyads[b,c] III *LEU2* +:−	IV *TRP1* +:−	V *URA3* +:−	*MAT* segregation **a**:α	N:α, N:**a**	N:N
msh4 spo13-Δ4 / *msh4 spo13-Δ4*	119	87	13 (15%)	8 (9%)	7 (8%)	0 (<2%)	13 (15%)	74 (85%)
msh4 spo13-23 / *msh4 spo13-23*	220	57	29 (51%)	34 (60%)	31 (54%)	20 (35%)	9 (16%)	28 (49%)

[a] Total spore viability in this experiment was 87% for *msh4 spo13-Δ4* and 52% for *msh4 spo13-23*.
[b] Reductional/aberrant segregation in dyads with two viable spores.
[c] The total frequency of aberrantly or reductionally segregating chromosomes in the genome (per cell/meiosis) is estimated to be ~9/16 chromosomes for *spo13-23* and 2/16 chromosomes for *spo13-Δ4*. This was calculated by multiplying the average +:− segregation for the three chromosomes monitored in each strain (55% for *spo13-23* and 11% for *spo13-Δ4*) by the total number of chromosomes in the genome (16). Reductional segregation only (excluding aberrant segregation) occurs (per cell/meiosis) for ~6 chromosomes in *spo13-23* and <1 chromosome in *spo13-Δ4* strains (based on the frequency of **a**:α segregation × 16 chromosomes).

functional) allele. In all cases, reductional division is dependent on the presence of recombining homologs (Figure 7B). The *spo13-9* and *spo13-10* alleles were sequenced and the mutations were found to reside in the *SPO13* open reading frame (Figure 7A). *spo13-9* contains a single point mutation (C to T), which changes a glutamine to stop codon, predicted to eliminate the last 72 amino acids of the 291-amino acid protein. Since this alteration allows partial function, the C-terminal portion of Spo13 does not appear to be absolutely essential for function. *spo13-10* contains a single point mutation (C to T), which results in a proline to alanine change at amino acid 136. These results demonstrate that lesions in the coding region similar to a promoter mutant produce a "reduced function" *spo13* phenotype that can be compensated for by recombination.

DISCUSSION

To achieve reductional segregation, in which homologs and not sisters separate at MI, cells must (1) keep sister centromeres together and (2) direct segregation of homologous chromosomes to opposite poles. The faithful separation of homologs away from each other will be referred to as "accurate homolog disjunction" and two sister chromatids segregating to the same pole will be referred to as "reductional chromosome behavior." In this study we define a new role for recombination

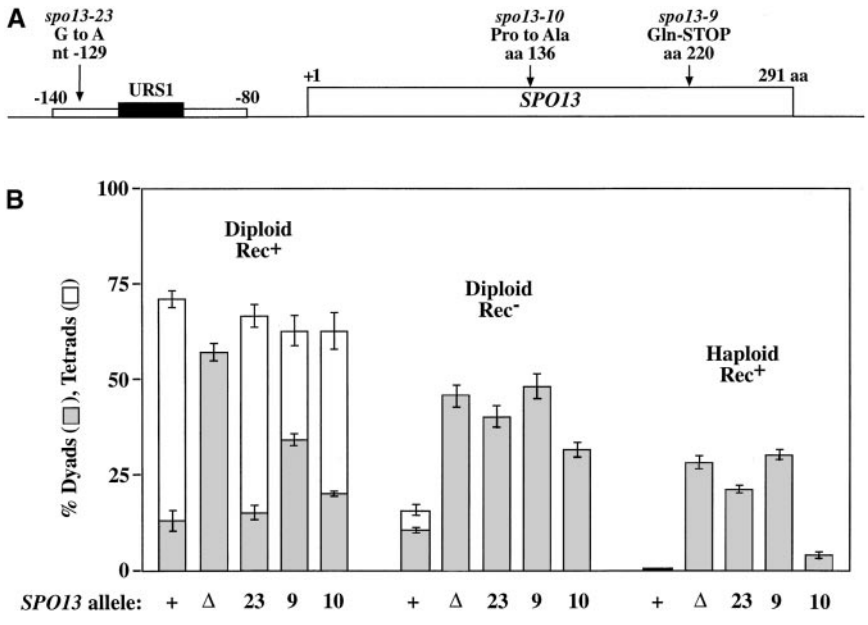

Figure 7.—Location and phenotypes of *spo13-9* and *spo13-10* alleles. (A) The *spo13-9* mutation leads to a glutamine to stop change at amino acid 220. The *spo13-10* mutation leads to a proline to alanine change at amino acid 136. (B) Graph showing production of dyads (filled bars) and tetrads (open bars) in Rec^+ diploids, Rec^- (*spo11*) diploids, and Rec^+ haploids in *SPO13*, *spo13-Δ4*, *spo13-9*, *spo13-10*, and *spo13-23* strains listed in Table 1.

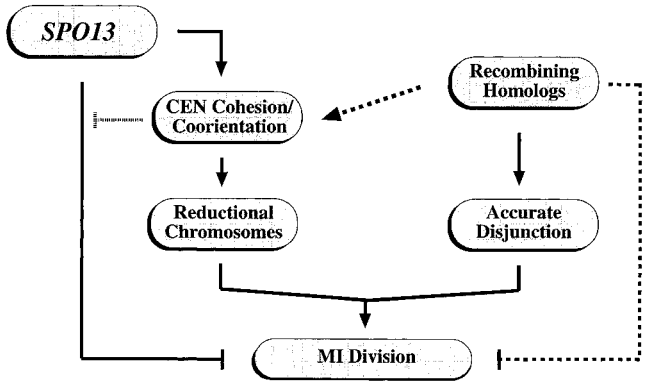

Figure 8.—The roles of *SPO13* and recombining homologous chromosomes in reductional segregation. *SPO13* is thought to promote reductional division by delaying the onset of nuclear division until sister chromatid cohesion/coorientation is properly established. Recombination brings homologous chromosomes together to ensure they segregate accurately from each other during MI. This model proposes that recombination between homologous chromosomes also promotes segregation of sisters to the same pole. Two possible pathways for this activity are shown with dashed lines. First, recombining chromosomes may act directly by promoting sister chromatid cohesion/coorientation. Alternatively, recombining chromosomes may act more indirectly, delaying nuclear division until other factors act to stabilize sister cohesion between sisters for their separation to the same pole at MI.

in MI segregation. In addition to its classically known function in directing accurate homolog disjunction, we show here that it also promotes reductional chromosome behavior. This conclusion is based on the finding that recombination can compensate for reduced function of *SPO13*, a gene known to regulate reductional chromosome behavior in meiosis.

Our present work provides an explanation for several prior results. For example, aberrant segregation (one chromosome reductional, one chromosome equational for a given homolog) in *spo13* mutants was shown to occur in Rec^+ but at greatly reduced levels in Rec^- strains (Klapholz and Esposito 1980b; Esposito and Klapholz 1981). This result can now be understood in the light of our current findings. In the absence of *spo13*, recombination causes some cohesion/coorientation of sister pairs leading to reductional chromosome behavior seen as aberrant segregation at the meiotic division.

How does recombination partially substitute for the reduction function of *SPO13*? A model for *SPO13* and recombinational control of reductional chromosome behavior at MI is shown (Figure 8). According to this model, *SPO13* ensures that sister chromatids segregate to the same pole at the first meiotic division by directly or indirectly promoting cohesion and/or coorientation of sister centromeres. As described earlier, it has been proposed that *SPO13* delays nuclear division until sister centromeres establish stable cohesion, after which the division delay is released and progression through M phase proceeds. Independently, recombining chromosomes align homologs for their separation from one another via the formation of chiasmata that provide necessary tension for proper microtubule attachment, thereby directing accurate homolog disjunction. Two possibilities are proposed (dashed lines) for how recombining homologs may also contribute to the pathway that directs sister centromeres to the same pole. In one, recombination acts in parallel to *SPO13*, providing a signal to delay division and to allow time for stable sister centromere association throughout the nucleus. In the other, recombination acts more directly (*in cis*) by physically constraining sister centromeres to coorient to the same pole. Although the precise mechanism by which recombination acts to promote reductional chromosome behavior is not yet clear, our recent work supports the view that a diffusible signal is involved. This is based on the demonstration that a single pair of recombining homologous chromosomes (in a haploid with either two copies of chromosome III or a homologous pair of artificial chromosomes) can promote reductional behavior of other chromosomes in the nucleus (L. H. Rutkowski and R. E. Esposito, unpublished results). Consistent with the results of the study described here, the level of reductional segregation in these strains is not sufficient to trigger two meiotic divisions.

As discussed above, recombination can promote reductional chromosome behavior when *SPO13* function is reduced. However, when *SPO13* is absent, although it does promote some reductional chromosome behavior (*e.g.*, aberrant segregation), it is not sufficient to trigger reductional segregation throughout the whole nucleus. This suggests that *SPO13* is required for the major pathway promoting sister centromere cohesion and that recombination plays an important, but secondary, role.

Does recombination act to delay meiotic division, similar to the proposed behavior of *SPO13*? A number of studies have provided evidence for some recombinational control of the timing of the meiotic divisions. For example, MI segregation in specific Rec^- null mutants defective in double-strand break formation (*spo11*, *rec104*, *rec114*, *rec102*, and *rad50*) has been reported to occur significantly earlier than in wild-type strains (Klapholz *et al.* 1985; Galbraith *et al.* 1997; Jiao *et al.* 1999). These results suggest that initiation of recombination normally creates a transient delay in the onset of MI, presumably allowing time for completion of recombination. In addition, dependent on strain background, mutants in other Rec genes that normally function after double-strand break formation (*e.g.*, *DMC1*, *ZIP1*, and *HOP2*) arrest in pachytene (Bishop *et al.* 1992; Sym *et al.* 1993; Leu *et al.* 1998). In these mutants, the presence of unresolved recombination intermediates acts through a pachytene checkpoint to prevent the onset of meiosis I. The meiotic arrest requires the mitotic checkpoint genes *RAD17*, *RAD24*, and *MEC1* in *dmc1* mutants (Lydall *et al.* 1996), and *PCH2* in both *zip1*

and *dmc1* mutants (San-Segundo and Roeder 1999). Phosphorylation of the *CDC28* cyclin-dependent kinase by another checkpoint gene, *SWE1*, is also required for the pachytene checkpoint arrest in *dmc1*, *zip1*, and *hop2* mutants (Leu and Roeder 1999). These studies collectively suggest that the pachytene delay or arrest caused by double-strand breaks and the presence of unresolved recombination intermediates may play a similar role to *SPO13* in delaying MI and providing time for stabilization of sister centromere cohesion.

Strikingly, studies of the *msh4* and *msh5* mutants indicate that both pathways for the action of recombination depicted in the model must depend not only on the initiation of exchange, but also on completed crossover events, presumably chiasmata. The only major defect in these mutants is a reduction in the levels of crossovers, which vary from near normal in some regions to only 30% of wild type in other regions (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995). If the MI-delaying function of recombination, described above, is responsible for suppressing the *spo13-23* phenotype, then the fact that *msh4* and *msh5* are required for the suppression suggests that even the resolution process can trigger MI delay. It is now known that the level of crossovers per chromosome is exquisitely regulated, with small chromosomes having a significantly higher frequency of recombination per kilobase as compared to larger ones (Kaback *et al.* 1989, 1999), ensuring at least one crossover per chromosome to promote accurate reductional disjunction. Assuming that at least one crossover event is also needed for an individual chromosome to segregate with sisters together (reductional chromosome behavior) when Spo13 is limiting, then an alternative possibility is that the *msh4* and *msh5* reduction in reciprocal exchange leaves some chromosomes with no chiasmata. Thus, even a moderate reduction in crossing over in *msh4* and *msh5* mutants (~30% of wild type) can have a highly significant effect, producing chromosomes that segregate equationally.

The intriguing finding that *msh4 spo13-23* mutants exhibit significant levels of reductional segregation, yet still produce predominantly dyads, raises the question of how many chromosomes must segregate in an accurate reductional division for a second meiotic division to be triggered. If results observed for the three chromosomes whose segregation was tested in *msh4 spo13-23* sporulation are extrapolated to the entire genome, we would predict approximately six chromosomes to segregate reductionally, approximately three aberrantly, and the remaining approximately seven equationally in each meiotic nucleus undergoing dyad formation (see Table 3, footnote c). This implies that more than half the genome must exhibit reductional chromosome behavior for two meiotic divisions to occur when Spo13 is limiting. Alternatively, even one chromosome segregating aberrantly might be sufficient to trigger a checkpoint control preventing two meiotic divisions. The precise mechanism by which the level of reductional *vs.* equational segregation is monitored remains to be determined. Recent evidence suggests that the *MAD2* gene may play a role in this monitoring mechanism since in the absence of Mad2, *spo13*Δ diploids complete two meiotic disivions (M. A. Shonn, R. McCarroll and A. Murray, personal communication).

Another interesting question is whether crossover position influences the level of reductional segregation and hence the switch from dyads to tetrads when Spo13 is limiting. Using artificial chromosomes, it has been reported that crossing over near the centromere is, in fact, more effective at ensuring accurate homolog disjunction than recombination at other chromosomal locations (Ross *et al.* 1996). However, it is not yet known whether the position of crossing over is similarly important for recombining homologs to promote reductional chromosome behavior independent of accurate disjunction.

How do *SPO13* and sister cohesion factors direct meiotic division? As discussed earlier, *SPO13* is thought to promote sister centromere interactions needed for reductional segregation. A key feature of *spo13* meiosis is that while sister centromeres separate at the single meiotic division, chromatids do not segregate randomly, but instead disjoin from one another in a single, largely equational division (Klapholz and Esposito 1980b). The fact that sister chromatids separate from one another with fairly high fidelity implies the presence of *SPO13*-independent sister chromatid arm associations that prevent random segregation. Accordingly, since the wild-type Spo13 protein is presumed to be specifically required for centromere cohesion, independent factor(s) must control arm cohesion.

In previous sections, we discussed in detail a model for *SPO13* control of centromere cohesion based on the indirect division delay. A direct role for *SPO13* in promoting the sister chromatid cohesion necessary for reductional division was thought to be unlikely, since reductional segregation can occur in the absence of *SPO13*. For example, *spo13* null mutants exhibit reductional division in certain strain backgrounds and when sporulated under conditions that slow meiotic division (Hollingsworth and Byers 1989; Hugerat and Simchen 1993; McCarroll and Esposito 1994). However, since the present study revealed that *SPO13* and recombining homologous chromosomes may have redundant roles in promoting reductional chromosome behavior, the issue of whether *SPO13* may also act more directly in centromere cohesion needs to be reexamined. For example, the presence of reductional division in the absence of *SPO13*, noted above, could be due to recombination. This appears to be the case. For example, the single, largely reductional division seen in *spo13* null mutants in some backgrounds is recombination dependent (Hollingsworth and Byers 1989). In addition, preliminary experiments suggest that suppression of the *spo13* mutant phenotype by sporulation at

low temperature also depends on recombination (L. H. Rutkowski and R. E. Esposito, unpublished observations). Recent studies demonstrating a requirement for *SPO13* in localization of the meiotic cohesin Rec8 during the meiotic divisions are consistent with either a direct and/or an indirect role for *SPO13* in centromere cohesion (Klein *et al.* 1999).

What is the relationship of *spo13* to other mutants that exhibit a single equational division during meiosis? The current work included an extensive search for genomic mutations allowing haploids that express both mating type alleles to enter meiosis and produce viable spores resulting from a single equational segregation. A total of 10 new mutations in *SPO13* were isolated, but no mutations in other genes were uncovered. Thus, it was concluded that *spo13* mutations are likely unique in their ability to allow haploids to complete meiosis and produce viable products by eliminating reductional segregation. Mutations in *spo13* also appear to be unique in their ability to rescue Rec$^-$ mutants from lethality. However, *spo13* is not novel in bypassing reductional segregation (without disrupting either recombination or other events in sporulation). *spo12* and *slk19* mutants also enter meiosis and produce dyads with diploid spores resulting from a single meiotic division in which most chromosomes segregate equationally (Klapholz and Esposito 1980b; W. Saunders, personal communication). In contrast to *spo13*, they require recombining homologs to produce viable meiotic products. These findings suggest that sister centromeres are initially cohered and that the wild-type alleles of these genes act after the point at which recombining chromosomes (chiasmata) are required for proper spindle microtubule attachment. Since these mutants eventually undergo a single equational division, they must bi-orient sister centromeres to face opposite poles for proper attachment, enabling separation of sister chromatids at MII. The fact that recombining chromosomes are not required for *spo13* equational division implies that sister centromeres must become bi-oriented in this mutant prior to the point at which chiasmata are normally required for proper attachment. Accordingly, we postulate that *SPO13* acts early, promoting sister centromere cohesion/coorientation, and that *SPO12* and *SLK19* act later, after initial sister centromere cohesion, perhaps in promoting proper spindle attachment. In fact, three lines of experimental evidence support this idea. First, *SPO13* is transcriptionally induced in meiosis before *SPO12*. *SPO13* is induced as part of the early meiotic expression class while *SPO12*, which is already transcribed in mitosis (Parkes and Johnston 1992; Cho *et al.* 1998), is further upregulated as part of the middle meiotic expression group (S. Frackman and R. E. Esposito, unpublished results; Malavasic and Elder 1990; Chu *et al.* 1998). Second, *SPO13* is genetically epistatic to *SPO12*. *spo12 spo13* double mutants produce viable spores in both recombination-defective (Esposito and Klapholz 1981) and haploid meiosis (Wagstaff *et al.* 1982). Third, recent data support a role for *SLK19* (with mutant behavior similar to *spo12*) in spindle microtubule stabilization (Zeng *et al.* 1999), which may promote attachment of chromosomes to the spindle. In contrast to the above, recent work has shown that a high-copy *SPO13* plasmid can partially restore tetrad production to *spo12* mutants (and vice versa; Grether and Herskowitz 1999; B. Washburn, L. H. Rutkowski and R. E. Esposito, unpublished results), suggesting that each protein may play multiple roles in both centromere cohesion and spindle attachment that partially overlap with one another. Further studies on the nature of the interactions between *SPO13*, *SPO12*, and *SLK19* should help elucidate the functional relationship between cohesion and spindle attachment.

What promoter elements are important for meiosis-specific transcription of *SPO13*? This work identified a novel allele, *spo13-23*, with a mutation in the 5′ untranslated region that reduces expression of the *SPO13* mRNA to about half of wild-type meiotic levels. The location of the mutation and its effect on mRNA levels are interesting in light of previous work on the *cis*-elements needed for *SPO13* expression and suggest that *spo13-23* may help to define a new *SPO13* promoter element. Previously, our laboratory identified a 60-bp region upstream of *SPO13* from −80 to −140 needed for full levels of meiosis-specific expression of a *spo13-lacZ* fusion (Buckingham *et al.* 1990). This region contains the regulatory sequence URS1, which is bound by Ume6, a C_6 zinc cluster protein that controls both mitotic repression and meiotic activation of *SPO13* transcription (Strich *et al.* 1994; Anderson *et al.* 1995; Steber and Esposito 1995). Although the *SPO13* URS1 is sufficient for mitotic repression, it is not sufficient for full levels of meiotic activation. Since the *SPO13* promoter lacks the early meiotic activation sequence, UAS_H, first identified upstream of *HOP1* (Vershon *et al.* 1992), it has been thought that an additional meiotic activation sequence must be located within the 60-bp regulatory region (L. Buckingham and R. E. Esposito, unpublished results). The G to A (−129) mutation responsible for *spo13-23* reduced expression may prove useful in defining the promoter element(s) as it lies within the expected region upstream of URS1.

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