Proc. Nat. Acad. Sci. USA
Vol. 71, No. 8, pp. 3172–3176, August 1974

Genetic Recombination and Commitment to Meiosis in *Saccharomyces*

(meiosis/recombination)

ROCHELLE E. ESPOSITO AND MICHAEL S. ESPOSITO

Erman Biology Center, Department of Biology, University of Chicago, Chicago, Illinois 60637

Communicated by Herschel L. Roman, June 10, 1974

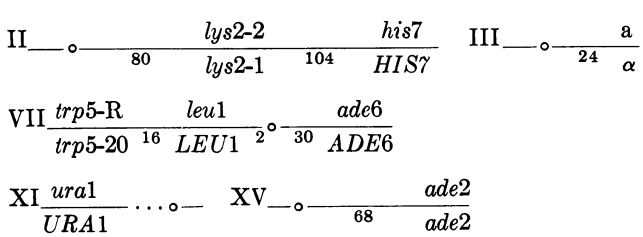
ABSTRACT **Diploid cells of the yeast *Saccharomyces cerevisiae* become committed to recombination at meiotic levels without becoming committed to the meiotic disjunction of chromosomes. These two events of the meiotic process can be separated by removing cells from a meiosis-inducing medium and returning them to a medium that promotes vegetative cell division. Cells removed at an appropriate time remain diploid, revert to mitosis, and display recombination with meiotic-like frequencies. Those removed after this time are committed to the completion of meiosis. Diploids of three conditional sporulation-deficient mutants (*spo*1-1, *spo*2-1, and *spo*3-1) have been examined for recombination at restrictive temperatures. All exhibit commitment to recombination without commitment to meiotic disjunction as in the wild type. Cells of *spo*1-1/*spo*1-1 do not replicate the spindle pole body for meiosis I; thus, recombination ability can be acquired by cells that do not proceed beyond this cytological stage.**

Diploid cells of *Saccharomyces cerevisiae* can be induced to undergo meisosis and ascospore formation (sporulation) by transfer from growth medium to sporulation medium. Several hours after this transfer, cells become committed to sporulation and do not revert to vegetative cell division when they are returned to growth medium (1). Premeiotic DNA synthesis and commitment to intragenic recombination (gene conversion) are initiated before cells become committed to meiosis (2, 3).

In this study we have examined both intragenic and intergenic recombination in a normal diploid strain and in three conditional sporulation-deficient mutants (*spo*1-1, *spo*2-1, and *spo*3-1). We provide evidence that commitment to intergenic as well as intragenic recombination at meiotic levels precedes commitment to meiotic chromosome disjunction and ascospore formation.

METHODS

Strains. Homothallic strains of *Saccharomyces cerevisiae* were used in this study. The genotype of the normal strain (Z193) and the relevant map distances (4) are given below:



*URA*1 was located on chromosome XI by trisomic analysis and displays no centromere linkage (4). The symbols are as follows: *a* and *α*, mating type alleles; *ade*2 adenine requiring; *his*7, histidine requiring; *leu*1, leucine requiring; *lys*2, lysine requiring; *trp*5, tryptophan requiring; *ura*1, uracil requiring. Roman numerals indicate chromosome number.

The genotypes of the three mutant diploids are identical to that of Z193 except that each is homozygous for a temperature-sensitive mutation preventing sporulation at 34°: C816, *spo*1-1/*spo*1-1; C817, *spo*2-1/*spo*2-1; and C819, *spo*3-1/*spo*3-1. Procedures for sporulation, dissection, and testing of auxotrophic requirements have been reported (5, 6).

Giemsa Staining and Counting of Asci. The appearance of asci was determined by hemocytometer counts of samples fixed in 4% formaldehyde. Giemsa staining was performed as described (7).

Allelism Tests. Diploids were tested for their allelic composition at *trp*5 and *lys*2 as follows. Strains were grown on solid nutrient medium, replica plated to sporulation medium, and incubated at 20° (a permissive temperature for *spo* diploids) for 5 days. The sporulation plate was then replica-plated to tryptophanless or lysineless medium, and growth was scored after 2 days at 30°. Due to genetic recombination during sporulation, replicas of heteroallelic diploids (*lys*2-1/*lys*2-2 or *trp*5-R/*trp*5-20) yield 50–100 prototrophs on either tryptophanless or lysineless medium, whereas homoallelic diploids (*lys*2-1/*lys*2-1, *lys*2-2/*lys*2-2, *trp*5-R/*trp*5-R, or *trp*5-20/*trp*5-20) yield few or none.

RESULTS

The Kinetics of Intragenic Recombination in Z193. Intragenic recombination at the *lys*2 and *trp*5 loci was monitored during sporulation of Z193. Cells were introduced into liquid sporulation medium at 34°. Samples were removed at intervals and plated on synthetic complete, lysineless, and tryptophanless medium. All plates were incubated at 34°, and colonies were counted after 5–7 days of growth. The appearance of asci in cultures was determined by microscopic examination of formalin-fixed samples withdrawn at the time of plating.

The data are shown in Fig. 1. Lysine and tryptophan prototrophs in excess of the starting background are recovered about 6 hr after introduction of the cells into sporulation medium. The maximum yield of prototrophs is obtained by 30 hr.

Commitment to Meiosis in Z193. As mentioned earlier, cells committed to meiosis and spore formation complete these processes when they are returned to vegetative growth medium. In homothallic strains like Z193, the haploid segregants diploidize during the early divisions after spore germination.

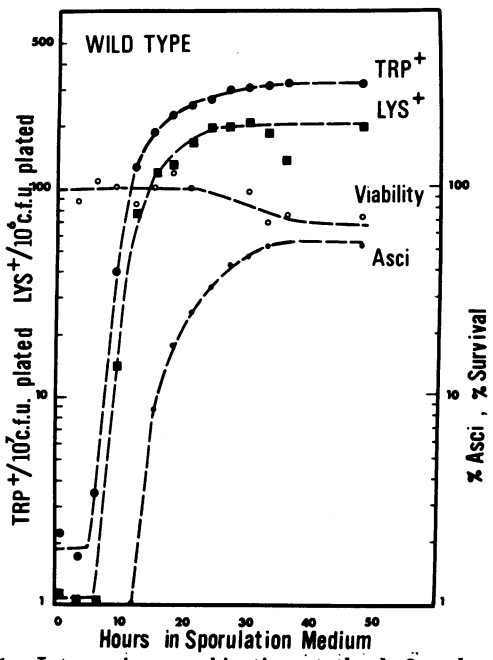


FIG. 1. Intragenic recombination at the *lys2* and *trp5* loci after exposure of diploid Z193 to sporulation medium at 34°. Cells withdrawn at the times indicated were plated on synthetic complete medium to assay viability and on lysineless and tryptophanless media to detect prototrophic intragenic recombinants. Prototroph values are given per colony forming units (*c.f.u.*) plated. Ascus production was observed by microscopic examination of samples fixed in formalin.

All intragenic recombinants are recovered as diploid clones, but a distinction can be made between those derived from uncommitted cells and those derived from cells committed to meiosis if the starting culture has the appropriate genotype. Intragenic recombinants resulting from the growth of uncommitted diploids remain heterozygous for loci closely linked to their centromeres except when recombination renders one or more loci homozygous. Cells committed to meiosis give rise to recombinant clones that are homozygous at all loci except for the mating type locus, which becomes heterozygous during diploidization (8).

The procedure used to detect committed cells among lysine prototrophs is illustrated in Fig. 2. Z193 is heterozygous for *leu1* located 2 map units from the centromere of chromosome VII. Since *leu1* and *lys2* are located on different chromosomes and segregate independently, *leu1/leu1* homozygotes represent half of the lysine prototrophs resulting from the growth of committed cells. Therefore, twice the percent of leucine auxotrophs was taken to represent the fraction of committed cells among lysine prototrophs.

Fig. 3 shows the percentage of lysine prototrophs committed to meiotic chromosomal segregation assayed by this procedure. At each time, 50–100 lysine prototrophs were tested for leucine auxotrophy. It can be seen that nearly all of the prototrophs recovered at early times are uncommitted to meiosis (compare T9). This result indicates that commitment to intragenic recombination occurs before commitment to meiosis. The percentage of cells at or beyond anaphase I of meiosis was determined by Giemsa staining and is also shown in Fig. 3. Since cells committed to meiosis are detected before the completion of meiosis I, commitment to meiosis must occur before the end of the first division of meiosis.

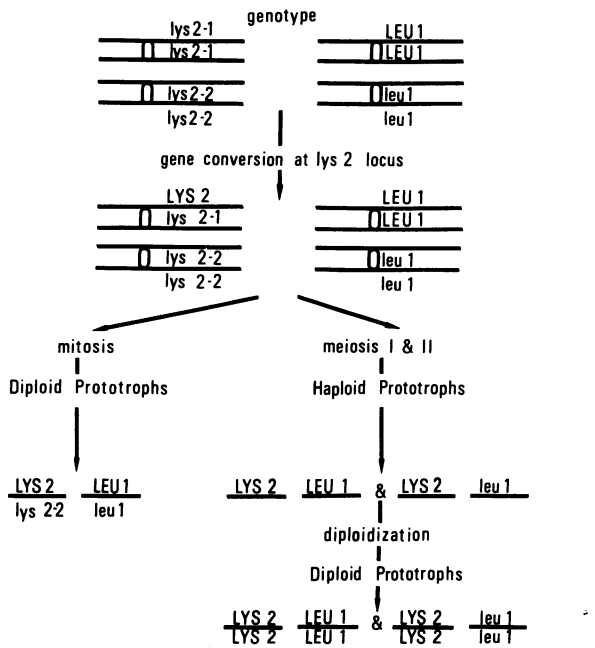


FIG. 2. Detection of cells committed to meiosis. Cells that undergo gene conversion at *lys2* and revert to mitosis on selective growth medium give rise to diploid colonies heterozygous for *lys2* and heterozygous for markers closely linked to their centromeres (e.g., *leu1*). Cells committed to meiosis give rise to diploid colonies homozygous for *LYS2* (wild type). By independent assortment, half of the committed lysine prototrophs are *leu1/leu1* and half are *LEU1/LEU1*. Twice the percent of leucine auxotrophs in a sample of lysine prototrophs is thus used to estimate the fraction of prototrophs representing the growth of cells that were committed to meiosis at the time of plating.

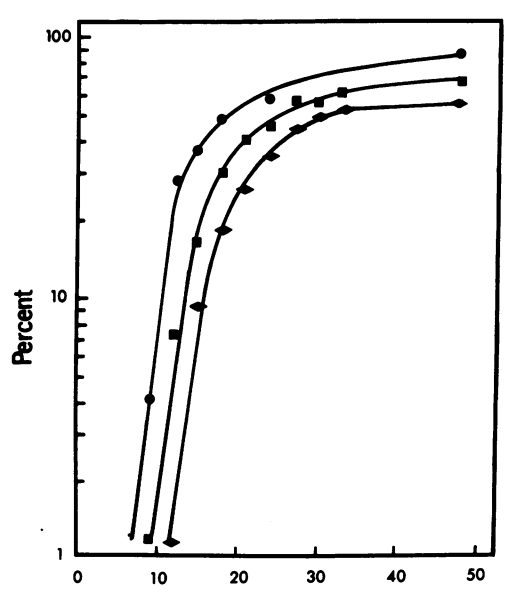


FIG. 3. Commitment to meiosis among lysine prototrophs and meiotic chromatin segregation during sporulation of diploid Z193. The percent of leucine auxotrophs observed × 2 (●) was used to estimate the percent of lysine prototrophs committed to meiosis as described in the *legend* of Fig. 2. Cell samples withdrawn from the sporulation culture were Giemsa stained to detect chromatin segregation in cells at or beyond anaphase of the first meiotic division (■). Ascus formation was monitored by visual examination (◆).

TABLE 1. *Commitment to meiosis among prototrophs obtained from sporulation cultures at 34°*

Strain	Genotype	Hr of sporulation	Prototroph class	No. dissected	% Uncommitted to meiosis*	% Committed to meiosis†
Z193	$\frac{spo^+}{spo^+}$	9	LYS	51	98	2
			TRP	33	96	4
C816	$\frac{spo1\text{-}1}{spo1\text{-}1}$	9	LYS	37	100	0
			TRP	31	99	1
C817	$\frac{spo2\text{-}1}{spo2\text{-}1}$	21	LYS	60	93	7
			TRP	50	93	7
C819	$\frac{spo3\text{-}1}{spo3\text{-}1}$	9	LYS	49	95	5
			TRP	40	92	8

* Uncommitted cells are heterozygous for *leu*1 and heterozygous at the locus where gene conversion occurred (*LEU*1/*leu*1 *LYS*2/*lys*2 or *LEU*1/*leu*1 *TRP*5/*trp*5).

† Committed cells are homozygous for *leu*1 and homozygous at the locus where gene conversion occurred (*leu*1/*leu*1 *LYS*2/*LYS*2; *LEU*1/*LEU*1 *LYS*2/*LYS*2; *leu*1/*leu*1 *TRP*5/*TRP*5; *LEU*1/*LEU*1 *TRP*5/*TRP*5).

Lysine and tryptophan prototrophs were sporulated at 20° and asci were analyzed to confirm the observations from replica plating that prototrophs initially represent the growth of diploid cells uncommitted to meiosis. Two distinct segregation patterns were observed. Prototrophs were either (*1*) heterozygous at the *leu*1 locus, heterozygous at the site of intragenic recombination, and heterozygous for other genetic markers except when gene–centromere recombination occurred, or (*2*) homozygous at the *leu*1 locus, homozygous wild type at the site of intragenic recombination, and homozygous for all other nutritional markers (Table 1). Prototrophs displaying the first segregation pattern represent cells that reverted to mitosis. Those displaying the second pattern represent cells that were committed to meiotic disjunction. Nearly 100% of lysine and tryptophan prototrophs recovered from cells plated at 9 hr in sporulation medium consist of cells uncommitted to meiosis, as expected from the replica plating experiment.

TABLE 2. *Alleles recovered among diploid prototrophs uncommitted to meiosis**

Strain	Genotype	LYS prototrophs: No. tested	LYS prototrophs: *LYS2*/*lys2*-1	LYS prototrophs: *LYS2*/*lys2*-2	TRP prototrophs: No. tested	TRP prototrophs: *TRP5*/*trp5*-R	TRP prototrophs: *TRP5*/*trp5*-20
Z193	$\frac{spo^+}{spo^+}$	46	23	23	25	13	12
C816	$\frac{spo1\text{-}1}{spo1\text{-}1}$	36	14	22	29	18	11
C817	$\frac{spo2\text{-}1}{spo2\text{-}1}$	49	25	24	35	19	16
C819	$\frac{spo3\text{-}1}{spo3\text{-}1}$	43	21	22	34	14	20

* Prototrophs from Z193, C816, and C819 are from 9 hr; prototrophs from C817 are from 21 hr.

Recovery of Input Alleles among Intragenic Recombinants. Lysine and tryptophan prototrophs uncommitted to meiosis, as noted above, segregated 2+:2− for lysine and tryptophan, respectively. The auxotrophic segregants of these tetrads were further tested for allelism to the input parental alleles (see *Methods*). The results for Z193 are summarized in Table 2. The prototrophic recombinants were found to contain one or the other of the parental alleles. The absence of *lys*2-1, 2-2 and *trp*5-R, 5-20 double mutants is expected since previous studies have demonstrated that intragenic recombination resulting in a prototroph occurs primarily by single site conversion (nonreciprocal recombination) and infrequently by reciprocal exchange (9).

Gene–Centromere Exchange among Uncommitted Lysine and Tryptophan Prototrophs. Lysine and tryptophan prototrophs were further examined to determine whether gene–centromere exchange had occurred. A sample of prototrophs, including those that had been dissected (Table 1), were replica-plated to detect gene–centromere recombination leading to homozygosity for recessive markers of heterozygous loci, and homoallelism for heteroallelic loci. Gene–centromere recom-

TABLE 3. *Gene–centromere recombination among lysine and tryptophan prototrophs uncommitted to meiosis**

Strain	Genotype	LYS prototrophs: No. tested	LYS prototrophs: % Gene–centromere recombination† *ade*6 (30)	*his*7 (33)	*leu*1 (2)	*trp*5 (18)	*ura*1 (33)	TRP prototrophs: No. tested	TRP prototrophs: % Gene–centromere recombination† *ade*6 (30)	*his*7 (33)	*leu*1 (2)	*lys*2 (33)	*ura*1 (33)
Z193	$\frac{spo^+}{spo^+}$	99	14.7	16.7	2.1	7.0	23.4	62	14.0	18.1	0.0	8.8	29.3
C816	$\frac{spo1\text{-}1}{spo1\text{-}1}$	70	14.0	23.5	3.9	20.2	28.4	62	10.0	15.4	0.0	12.6	26.8
C817	$\frac{spo2\text{-}1}{spo2\text{-}1}$	189	32.0	20.5	4.9	14.1	25.3	158	14.0	2.3	3.4	12.1	29.0
C819	$\frac{spo3\text{-}1}{spo3\text{-}1}$	83	22.0	14.6	0.6	9.0	37.9	66	16.0	23.0	3.6	16.6	31.9

* Prototrophs from Z193, C816, and C819 are from 9 hr. Prototrophs from C817 are from 21 hr.

† Percent of gene–centromere recombination = (C − mT)/(T − mT) × 100. C = 2 × number of −/− homozygous diploids observed or total number of homoallelic diploids observed; m = fraction of meiotic diploids in the sample (Table 1); T = total colonies tested. Percent of gene–centromere recombination expected based on meiotic map values is given in *parentheses* below each marker.

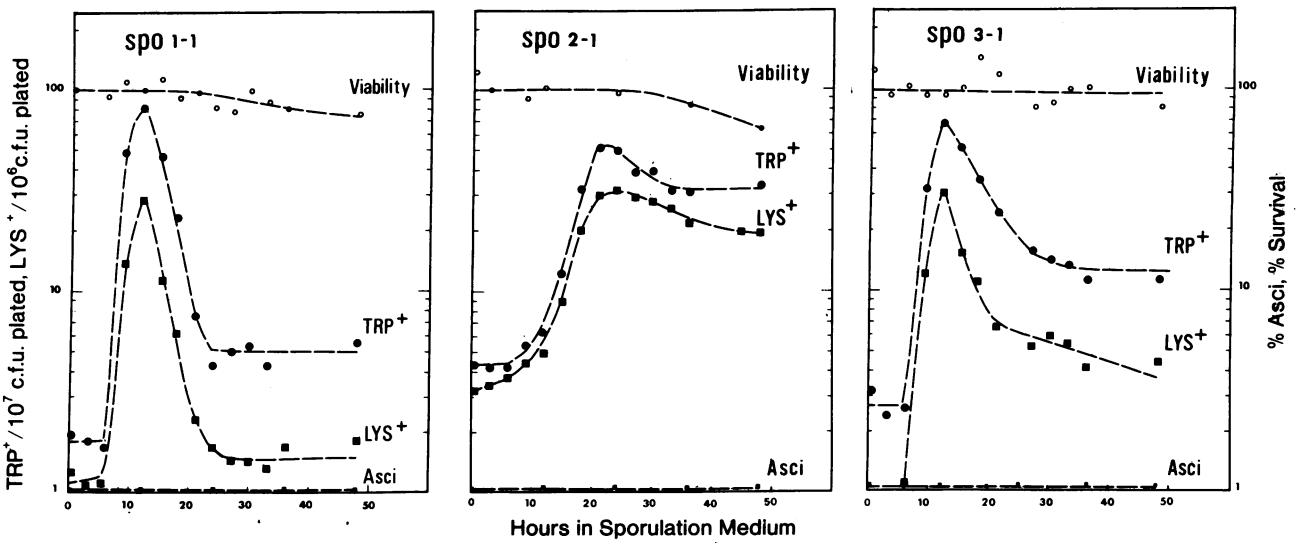


FIG. 4. Intragenic recombination at the *lys*2 and *trp*5 loci after exposure of *spo*1-1/*spo*-1 (C816), *spo*-2-1/*spo*2-1 (C-817), and *spo*3-1/*spo*3-1 (C819) to sporulation medium at 34°. Viability, prototroph production, and ascus formation were monitored as described in the *legend* of Fig. 1.

bination in the case of the *ade*6 locus was detected by a change in color of colonies plated on growth medium. The parental strain, homozygous for *ade*2 and heterozygous for *ade*6, forms red colonies whereas recombinant cells homozygous for *ade*2 as well as *ade*6 form white colonies (10).

The data for percent of gene–centromere recombination for each of the markers studied are shown in Table 3. The percentages of gene–centromere recombination among lysine and tryptophan prototrophs are orders of magnitude greater than those generally encountered in mitotic populations. Intergenic recombination among prototrophs is enhanced for markers located on different linkage groups as well as for markers located on the same chromosome where intragenic recombination occurred.

Exchange between the centromere and *leu*1, and the centromere and *ura*1, is at or near the meiotic level. Gene–centromere recombination for the markers *ade*6, *his*7, *lys*2, and *trp*5 occurs at frequencies that are less than the meiotic level.

Genetic Recombination in spo Mutants. Genetic recombination was examined in three temperature-sensitive, sporulation-deficient mutants that exhibit premeiotic DNA synthesis at the restrictive temperature (11). The appearance of prototrophs in cultures of *spo*1-1, *spo*2-1, and *spo*3-1 diploids is shown in Figure 4. The kinetics of prototroph recovery in *spo*1-1 and *spo*3-1 are similar to those of the wild type until 12 hr, after which time the number of prototrophs recovered declines. The appearance of prototrophs in *spo*2-1 is delayed. Prototrophs in this mutant are recovered with increasing frequency until 24 hr, and then the number recovered decreases.

A sample of lysine and tryptophan prototrophs recovered from cultures of *spo* mutant diploids was sporulated at 20° (permissive temperature). Tetrad analysis was performed to determine the fraction of prototrophs committed to meiosis, as described (Table 1). Greater than 90% of the prototrophs recovered from *spo*1-1 and *spo*3-1 at 9 hr and *spo*2-1 at 21 hr were uncommitted to meiosis.

Tetrad analysis revealed that the uncommitted prototrophs were heterozygous at the site of intragenic recombination. Upon sporulation at a permissive temperature, the prototrophs yielded asci with 2+:2− segregations. Like Z193, all of the auxotrophic spores contained only one of the parental alleles as expected from nonreciprocal recombination.

Gene–centromere exchange was monitored as described. The percent of gene–centromere recombination for each of the markers studied in diploids of *spo*1, *spo*2, and *spo*3 is given in Table 3. Intergenic recombination in each of the *spo* mutants studied is enhanced among cells uncommitted to meiotic disjunction.

DISCUSSION

The data reported above indicate that cells stimulated to enter meiosis become committed to intragenic and intergenic recombination before commitment to meiotic chromosome disjunction. Since cells that exhibit meiotic-like recombination frequencies in certain intervals can revert to mitosis, we must conclude that the events of chromosome pairing and exchange that occur in these cells are not sufficient to commit the cells to meiosis. Commitment to recombination at meiotic levels and commitment to meiotic disjunction are therefore separable processes.

While gene–centromere recombination among *lys*2 and *trp*5 prototrophs uncommitted to meiotic disjunction was at the meiotic level in certain intervals, it was below the meiotic level in others. This heterogeneity may be explained by assuming that different regions of the genome become available for or participate in recombination (i.e., commitment to recombination) in an ordered manner and that commitment to recombination is terminated prematurely when cells are returned to growth medium. Accordingly, prototrophic recombinants uncommitted to meiotic disjunction may exhibit a level of gene–centromere recombination elsewhere in the genome depending upon (*i*) the sequence with which these intervals become committed to recombination, relative to the time at which the region containing the heteroallelic locus becomes committed to recombination, and (*ii*) the time during sporulation when the selected recombinants are harvested.

Although our present data provide evidence that commitment to recombination begins before commitment to meiotic

disjunction, it is not yet known whether the commitment to exchange is completed in all genetic intervals before the commitment to meiotic disjunction.

Sporulation cultures of three sporulation mutants (*spo*1-1, *spo*2-1, and *spo*3-1) exhibited a decline in recovery of prototrophic recombinants with increasing time of incubation in sporulation medium. This decrease in prototroph recovery is consistent with the cytological properties of *spo*2-1 and *spo*3-1. Electron microscopy of these mutants has shown that the ascogenous cells form aberrant anucleate and partially anucleate ascospores that fail to mature (12). The decline in recombinant recovery observed in cultures of *spo*2-1 and *spo*3-1 thus probably reflects the loss of colony-forming ability by recombinant cells that become committed to aberrant sporulation. It should be noted that the viability of *spo* mutants incubated in sporulation medium at the restrictive temperature (Fig. 4) is overestimated since the cultures contain budded as well as unbudded cells.

Fine structural studies of *spo*1-1 diploids at the restrictive temperature have shown that *spo*1 terminates sporulation before formation of the spindle bodies for meiosis I (11). Since this mutant exhibits recombination after exposure to sporulation medium, duplication of the spindle pole body for meiosis I is not required for commitment to genetic recombination.

We thank Herschel Roman, Rodney Rothstein, Dale Fast, and Arnold Ravin for helpful criticisms of the manuscript, and Norman Frink for excellent technical assistance. This research was supported by NSF Grants GB-8465, GB 27688, and by the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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