Nonrandom Homolog Segregation at Meiosis I in *Schizosaccharomyces pombe* Mutants Lacking Recombination

Luther Davis and Gerald R. Smith[1]

Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024

Manuscript received August 27, 2002
Accepted for publication December 5, 2002

ABSTRACT

Physical connection between homologous chromosomes is normally required for their proper segregation to opposite poles at the first meiotic division (MI). This connection is generally provided by the combination of reciprocal recombination and sister-chromatid cohesion. In the absence of meiotic recombination, homologs are predicted to segregate randomly at MI. Here we demonstrate that in *rec12* mutants of the fission yeast *Schizosaccharomyces pombe*, which are devoid of meiosis-induced recombination, homologs segregate to opposite poles at MI 63% of the time. Residual, Rec12-independent recombination appears insufficient to account for the observed nonrandom homolog segregation. Dyad asci are frequently produced by *rec12* mutants. More than half of these dyad asci contain two viable homozygous-diploid spores, the products of a single reductional division. This set of phenotypes is shared by other *S. pombe* mutants that lack meiotic recombination, suggesting that nonrandom MI segregation and dyad formation are a general feature of meiosis in the absence of recombination and are not peculiar to *rec12* mutants. Rec8, a meiosis-specific sister-chromatid cohesin, is required for the segregation phenotypes displayed by *rec12* mutants. We propose that *S. pombe* possesses a system independent of recombination that promotes homolog segregation and discuss possible mechanisms.

IN sexually reproducing organisms two gametes fuse to form a zygote, which then develops into an adult. To maintain a constant number of chromosomes from generation to generation, the gametes must contain precisely one-half the diploid number of chromosomes. Meiosis is the specialized form of cell division that accomplishes this task (Figure 1). A diploid cell undergoes meiotic DNA replication, followed by an extended prophase during which homologous chromosomes (homologs), each consisting of two sister chromatids, must find each other, align, synapse, and recombine. At the first meiotic division (MI) homologs segregate to opposite poles, accomplishing the most critical function of meiosis, reducing by half the number of chromosomes. For this reason MI is called a reductional division. The second meiotic division (MII), unlike mitosis and MI, is not immediately preceded by a round of DNA replication. However, like mitosis, MII segregates sister chromatids to opposite poles and is called an equational division. The net result of these processes is the production of four haploid progeny from one diploid cell.

Clustering of the ends of the chromosomes or telomeres, one of the earliest events in meiosis, facilitates the initial interaction between homologs (reviewed in Scherthan 2001; Yamamoto and Hiraoka 2001) and, in the fission yeast *Schizosaccharomyces pombe*, is required for wild-type levels of pairing and meiotic recombination (Shimanuki *et al.* 1997; Cooper *et al.* 1998; Nimmo *et al.* 1998; Yamamoto *et al.* 1999). In *S. pombe*, meiotic replication is thought to be required for wild-type levels of meiotic recombination and for the reductional nature of MI (Watanabe *et al.* 2001). Recombination is enhanced indirectly by these early processes. However, Rec12, like its homologs in other organisms, is thought to directly promote meiotic recombination by catalyzing the formation of meiotic DNA double-strand breaks (DSBs) via a topoisomerase-like mechanism (Bergerat *et al.* 1997; Keeney *et al.* 1997; Cervantes *et al.* 2000). The mutant protein Rec12(Y98F), in which a tyrosine at the presumptive catalytic site is replaced by a phenylalanine, differs from wild type only by the absence of the presumptive reactive oxygen atom. The *rec12-164* allele, which encodes Rec12(Y98F), is strongly recombination deficient (Cervantes *et al.* 2000).

Recombination is thought to be required for proper MI segregation in the following way (Figure 1): Proper segregation of homologs at MI requires that each homolog in a pair attach to spindle microtubules from opposite poles. Bipolar attachment produces tension when the poleward forces exerted by the spindle apparatus are opposed by a physical connection between homologous chromosomes. This tension stabilizes the spindle's attachment to the chromosomes, reinforcing the bipolar attachment (reviewed in Nicklas 1997). In most organisms, reciprocal recombination, or crossing over, in conjunction with sister-chromatid cohesion (SCC) provides the connection between homologous chromosomes. In

[1]*Corresponding author:* Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. North, A1-162, P.O. Box 19024, Seattle, WA 98109-1024. E-mail: gsmith@fhcrc.org

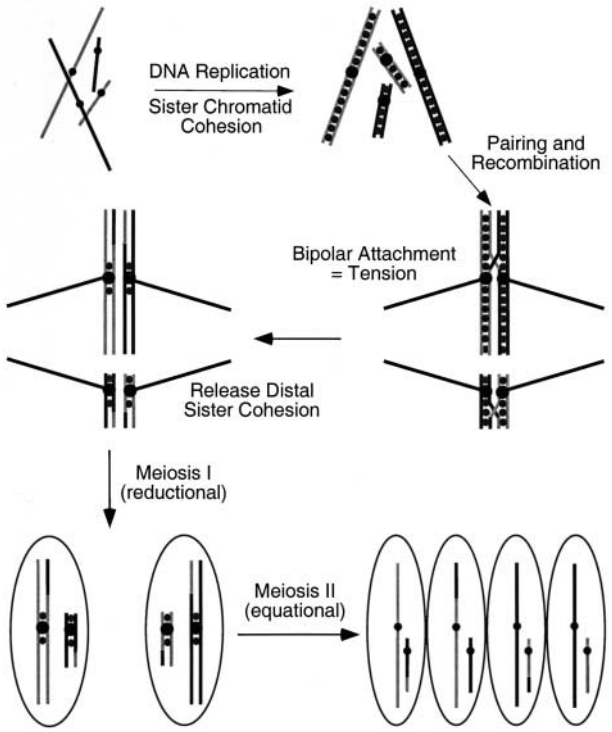


FIGURE 1.—Meiotic chromosome segregation. Meiosis consists of one round of DNA replication followed by an extended prophase. Prophase is followed by the first (reductional) meiotic division, which segregates homologous chromosomes to opposite poles, and by the second (equational) meiotic division, which segregates sister chromatids to opposite poles.

many organisms these connections are cytologically visible and are called chiasmata.

Meiotic chromosome segregation is initiated when tension signals the bipolar attachment of microtubules to each homolog pair. MI segregation is triggered by the release of SCC distal to the crossovers, disentangling the homologs and permitting them to segregate to opposite poles. However, centromere-proximal SCC is maintained until anaphase of MII to promote bipolar attachment of microtubules to sister chromatids. Finally, release of the remaining SCC triggers the segregation of sisters to opposite poles at MII.

In the budding yeast *Saccharomyces cerevisiae*, separin-dependent cleavage of Rec8, a meiosis-specific homolog of the sister-chromatid cohesin Scc1 (KLEIN *et al.* 1999; PARISI *et al.* 1999; WATANABE and NURSE 1999), is required for the segregation of recombined homologs at MI (BUONOMO *et al.* 2000). *S. pombe* Rec8 possesses a sequence that is homologous to separin cleavage sites (UHLMANN *et al.* 1999; NASMYTH 2001), suggesting that Rec8 cleavage may trigger the release of meiotic SCC in *S. pombe* also.

Meiotic chromosome missegregation results in aneuploid gametes and, eventually, aneuploid zygotes. Aneuploidy generated during meiosis is of considerable medical interest. Aneuploidy is associated with ~35% of lost pregnancies in humans (reviewed in HASSOLD and HUNT 2001). Aneuploidy, including trisomy 21, which results in Down syndrome, is the leading known cause of mental retardation. In humans, decreased meiotic recombination is associated with trisomy 21 and other MI missegregation events (reviewed in HASSOLD and HUNT 2001). Understanding the role of recombination in meiotic chromosome missegregation is therefore of great importance.

Mutants deficient in meiotic recombination may display several types of segregation defects (Figure 2). In the absence of the physical connection between homologs promoted by recombination, homologs are expected to segregate randomly at MI. In this case homologous chromosomes will move to the same pole in one-half of the MI divisions (Figure 2B). The absence of recombination could also perturb sister-chromatid interactions. Precocious separation of sister chromatids (PSSC) prior to MI results in random segregation of sister chromatids at MI. The chromatids may move either to opposite poles at MI (Figure 2C) or to the same pole, where they are free to segregate randomly again at MII (Figure 2D). Additionally, PSSC after MI but prior to anaphase of MII would allow sisters to segregate randomly at MII (Figure 2D). Although not shown, homologs and/or sister chromatids may also be lost (*i.e.*, not packaged into a spore).

While reciprocal recombination is usually required for proper MI homolog segregation, examples of recombination-independent, or achiasmate, segregation exist. Achiasmate chromosomes segregate properly during meiosis in the fruit fly *Drosophila melanogaster* (reviewed in HAWLEY 1989; HAWLEY and THEURKAUF 1993; MCKEE 1998). *S. cerevisiae* also has an achiasmate system capable of promoting the segregation of achiasmate artificial chromosomes (DAWSON *et al.* 1986) or two monosomic nonhomologous chromosomes (GUACCI and KABACK 1991). Additionally, data from the Kohli

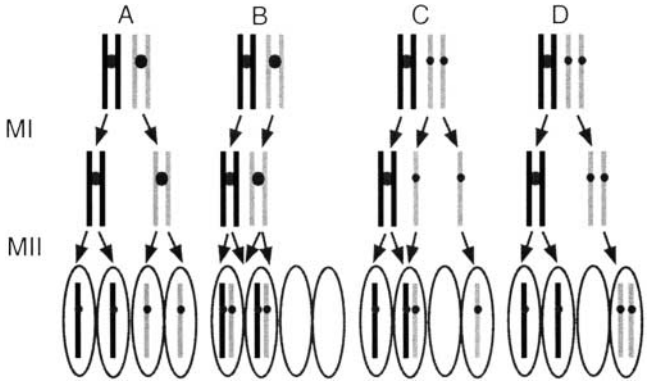


FIGURE 2.—Meiotic chromosome missegregation. (A) Proper segregation of one set of homologs, as in Figure 1. (B) Meiosis I homolog missegregation. (C and D) Precocious separation of sister chromatids can result in premature segregation of sister chromatids at MI (C) or missegregation at MII (D).

and Hiraoka labs (Molnar *et al.* 2001a,b) and in this article indicate that a mechanism exists in *S. pombe* that promotes proper MI segregation in the absence of meiotic recombination.

We chose *S. pombe* as an experimental organism to study the role of recombination in meiotic chromosome segregation for the following reasons: as in *S. cerevisiae*, and most likely Arabidopsis, *Caenorhabditis elegans*, Coprinus, Drosophila females, and mouse (reviewed in Lichten 2001), meiotic recombination in *S. pombe* is initiated by the formation of DNA double-strand breaks (Cervantes *et al.* 2000). In the absence of Rec12, which is essential for the formation of meiotic DNA double-strand breaks (Cervantes *et al.* 2000), meiotic recombination in *S. pombe* is reduced by as much as 1000-fold (DeVeaux *et al.* 1992; this article). Because *S. pombe* has only three chromosomes, mutants that randomly segregate homologs are expected to produce a significant number of viable progeny. This simple feature allows the genetic analysis of meiotic products characteristic of missegregation as well as the rapid identification of mutations that specifically affect segregation. Here we use both genetic and cytological assays to show that in the absence of meiotic recombination segregation of homologs to opposite poles at MI is significantly more frequent than random segregation would predict. Possible bases for this nonrandom segregation are discussed.

MATERIALS AND METHODS

Yeast strains, media, and culture conditions: The yeast strains used in this work are described in Table 1. Complete genealogies are available upon request. The *cyh1-101* allele used in this work was derived in this lab by selection on plates containing cycloheximide at a final concentration of 100 μg/ml. Tight linkage (1.6 cM; data not shown) to *lys1* confirmed the classification of the mutation as an allele of *cyh1*. Other mutations are described below or have been previously described.

Replacement of the *rec12* coding sequence with *3HA-6His-kanMX* was performed as follows: plasmid pFA6a-3HA-kanMX6 (Bahler *et al.* 1998) was modified to contain six histidine codons, using the Morph kit (5′–3′, Inc) and 5′-CGTTCCA GATTACGCTGCTCAGTGCCACCATCACCACCATCATTGA GGCGCGCCACTTCTAAATAAGCG-3′ as a replication primer. Candidate plasmids were screened by restriction digestion and confirmed by sequencing. Plasmid pFA6a-3HA-6His-kanMX6 was then used as template for the polymerase chain reaction (PCR) that created the *rec12* deletion construct. The deletion construct was used to transform *S. pombe* strain GP2232 (h^{90} *ade6-M216 leu1-32 ura4-D18 mes1::LEU2*) as described (Bahler *et al.* 1998). The resulting *rec12-169::3HA6His-kanMX* allele was confirmed by a PCR.

To integrate the *rec12-164* allele (Y98F; Cervantes *et al.* 2000) onto the chromosome, the *rec12-171::ura4*$^+$ deletion of the *rec12* coding sequence was created as follows: The *ura4*$^+$-containing *Hin*dIII fragment (Grimm *et al.* 1988) was amplified from plasmid pKS-ura4 (Bahler *et al.* 1998), and *Pme*I and *Bgl*II sites were added to the ends by a PCR. The PCR product was restricted with *Pme*I and *Bgl*II and ligated into the *Pme*I and *Bgl*II sites of pFA6a-kanMX6 (Bahler *et al.* 1998), creating pLD206. The same primers that are used with the pFA6a series of vectors to target integration of *kanMX6* (Bahler *et al.* 1998) can be used with pLD206 to target integration of *ura4*$^+$. This plasmid was used as template in a PCR to generate the *rec12-171::ura4*$^+$ allele using the method of Bahler *et al.* (1998). The resulting PCR product was used to transform GP2682 (h^+ *ade6-M216 lys1-37 leu1-32 ura4-D18 rec12-152::LEU2*) to Ura$^+$, and the transformants were screened for the loss of the *LEU2* marker. Among 50 Ura$^+$ transformants, 2 (GP3398 and GP3399) were Leu$^-$. Deletion of *rec12* was confirmed by PCR. The *rec12-164*-containing plasmid, pJF03 (Cervantes *et al.* 2000), was restricted with *Hin*dIII to release the *rec12* gene and used to transform GP3398 to Ura$^-$. The Ura$^-$ transformants were selected on supplemented EMM2 + 1 mg/ml 5-fluoroorotic acid (Grimm *et al.* 1988). The *rec12-164* (Y98F) replacement was confirmed by sequencing the entire coding sequence.

To enhance the signal from the *lacO*-tagged chromosome I (ChrI), a previously described variant of the green fluorescent protein (GFP)-LacI-nuclear localization signal (NLS) fusion with increased fluorescence, thermostability, and *lacO* binding (Straight *et al.* 1998) was adapted for *S. pombe*. Plasmid pAFS144 (Straight *et al.* 1998) was restricted with *Not*I and the ends were made blunt with the Klenow fragment of *Escherichia coli* DNA polymerase I. The 1.8-kb *Not*I-*Xho*I fragment containing GFP13-LacI12-NLS was ligated into the *Sma*I and *Xho*I sites of pREP41x (Forsburg 1993), placing GFP13-LacI12-NLS between the *nmt1* promoter and terminator and creating pLD203. Plasmid pLD203 was restricted with *Sph*I and the ends were made blunt with T4 DNA polymerase. The *nmt1p*-GFP13-LacI12-NLS-*nmt1t* cassette was released by restriction with *Sac*I and ligated into the *Not*I (made blunt with the Klenow fragment of *E. coli* DNA polymerase I) and *Sac*I sites of pBluescript KS+ (Stratagene, La Jolla, CA), creating pLD207. The *his7*$^+$ open reading frame (Apolinario *et al.* 1993) plus 500 bp of upstream and 212 bp of downstream sequence was amplified, and *Nar*I and *Kpn*I sites were added by a PCR using genomic DNA as template. The PCR product was restricted with *Nar*I and *Kpn*I and ligated into the *Cla*I and *Kpn*I sites of pLD207, creating pLD213. The *dis1*$^+$ promoter sequence (from −1 to −1012 relative to the ATG; Nabeshima *et al.* 1995) was amplified and *Xho*I and *Spe*I sites were added by PCR. The sites were used to replace the *nmt1* promoter with the *dis1* promoter, upstream of the GFP13-LacI12-NLS coding sequence, in pLD213. This created pLD214, which was linearized with *Cla*I and used to transform GP3420 (h^- *ade6-M210 leu1-32 lys1-37 his7-366 rec12-169::3HA6His-kanMX*). His$^+$ transformants were selected and correct integration confirmed by PCR.

Strains were grown on YEA + 4S [YEA (Gutz *et al.* 1974) plus 50 μg/ml histidine, 100 μg/ml leucine, 100 μg/ml lysine, and 100 μg/ml uracil], YEA + 5S (YEA + 4S plus 100 μg/ml adenine), or supplemented EMM2 (as modified in Nurse 1975) solid media at 32°. When needed, cycloheximide was added to YEA + 5S to a final concentration of 100 μg/ml. Liquid cultures were grown in YEL + 5S [YEL (Gutz *et al.* 1974) plus five supplements as in YEA + 5S] liquid media at 30°. Sporulation was at 25° on supplemented SPA (Gutz *et al.* 1974) for 2–4 days.

Calculation of the frequency of aneuploid meiotic products: We calculated the frequency of meiotic products that would occur at various levels of homolog missegregation at MI. For these calculations we assumed that (1) the only type of segregation error is MI homolog missegregation, (2) missegregation of homologs does not preclude microscopically visible spore formation, (3) all three homologs missegregate with equal frequency, and (4) ChrIII disomes are viable, while ChrI and ChrII aneuploids are inviable. The validity of these assumptions and the effect of deviations from them are considered in the discussion.

TABLE 1

***S. pombe* strains**

Strain	Genotype
GP720	*h⁻ ade6-52 ura4-294 leu1-32*
GP821	*h⁻ ade6-52 ura4-294 leu1-32 rec7-146::*Tn*10-LLK*
GP985	*h⁺ ade6-M26 leu1-32 ura4-294 rec7-146::*Tn*10-LLK*
GP1147	*h⁻ ade6-52 ura4-294 leu1-32 rec6-151::LEU2*
GP1253	*h⁺ ade6-M26 ura4-294 leu1-32 rec6-151::LEU2*
GP1456	*h⁻ ade6-52 leu1-32 ura4-294 rec12-152::LEU2*
GP1459	*h⁺ ade6-M26 leu1-32 ura4-294 rec12-152::LEU2*
GP1688	*h⁻ ade6-52 ura4-294 leu1-32 rec15-154::LEU2*
GP1825	*h⁺ ade6-M26 leu1-32 ura4-294 rec15-154::LEU2*
GP1994	*h⁺ ade6-M26 leu1-32 rec14-161::LEU2*
GP2004	*h⁻ ade6-52 leu1-32 rec14-161::LEU2*
GP2183	*h⁺ ade6-M26 ura4-294 leu1-32*
GP2656	Same as GP2659 except also *rec8::ura4⁺/rec8::ura4⁺*
GP2657	Same as GP2659 except also *rec12-152::LEU2/rec12-152::LEU2*
GP2658	Same as GP2657 except also *rec8::ura4⁺/rec8::ura4⁺*
GP2659	*h⁺/h⁻ ade6-M210/ade6-M216 leu-32/leu-32 ura4-D18/ura4-D18 lys1-37/+ cyh1-101/+*
GP3533	*h⁺ ade4-31 ura4-D18*
GP3534	*h⁺ ade4-31 ura4-D18 rec12-169::3HA6His-kanMX6*
GP3535	*h⁻ leu1-32 ura4-D18 spc1::ura4⁺*
GP3536	*h⁻ leu1-32 ura4-D18 spc1::ura4⁺ rec12-169::3HA6His-kanMX6*
GP3570	*h⁺ ade6-M26 ura1-161*
GP3571	*h⁺ ade6-M26 ura1-161 rec12-169::3HA6His-kanMX6*
GP3572	*h⁻ ade6-M210 leu2-120 pat1-114*
GP3573	*h⁻ ade6-M210 leu2-120 pat1-114 rec12-169::3HA6His-kanMX6*
GP3684	*h⁺ ade6-M216 lys1-37 rec12-164* (Y98F)
GP3712	*h⁻ ade6-M210 rec12-164* (Y98F)
GP3714	*h⁻ ade6-M210 lys1-37*
GP3715	*h⁻ ade6-M210 lys1-37 rec12-169::3HA6His-kanMX6*
GP3716	*h⁺ ade6-M216*
GP3717	*h⁺ ade6-M216 rec12-169::3HA6His-kanMX6*
GP3757	*h⁻ leu1-32 mes1::LEU2 lys1-37::lacO-lys1⁺ his7-366::GFP13-LacI12-NLS-his7⁺*
GP3758	Same as GP3757 except also *rec12-169::3HA6His-kanMX6*
GP3759	Same as GP3757 except *h⁺*
GP3760	Same as GP3758 except *h⁺*
GP3761	*h⁺ leu1-32 mes1::LEU2 lys1-37 his7-366::GFP13-LacI12-NLS-his7⁺*
GP3762	Same as GP3761 except also *rec12-169::3HA6His-kanMX6*
GP3764	*h⁺ ade6-M216 ura4-D18 lys1-37 rec8::ura4⁺*
GP3765	*h⁻ ade6-M210 ura4-D18 rec8::ura4⁺*
GP3766	Same as GP3764 except also *rec12-169::3HA6His-kanMX6*
GP3767	Same as GP3765 except also *rec12-169::3HA6His-kanMX6*

The strains used in this study were derived from lab stocks by genetic crosses. Complete genealogies are available upon request. Except as noted below, alleles are those commonly used (*i.e.*, auxotrophic markers, the mating-type locus, and *pat1-114*). The *cyh1-101* allele used in strains GP2656–GP2659 was derived in this lab as described in MATERIALS AND METHODS. Construction of the *rec12-164* (Y98F), *rec12-169::3HA6His-kanMX6*, and *his7-366::GFP13-LacI12-NLS-his7⁺* alleles is described in MATERIALS AND METHODS. Construction of the *rec6-151::LEU2*, *rec7-146::*Tn*10-LLK*, *rec12-152::LEU2*, *rec14-161::LEU2*, and *rec15-154::LEU2* alleles has been described previously (LIN *et al.* 1992; LIN and SMITH 1994, 1995; EVANS *et al.* 1997). The *rec8::ura4⁺* allele (PARISI *et al.* 1999) was a gift from Dr. Jürg Kohli. The *lys1-37::lacO-lys1⁺* allele (NABESHIMA *et al.* 1998) was a gift from Dr. Mitsuhiro Yanagida. The *mes1::LEU2* allele (KISHIDA *et al.* 1994) was a gift from Dr. Chikashi Shimoda. The *spc1::ura4⁺* allele (SHIOZAKI and RUSSELL 1995) was a gift from Dr. Paul Russell.

Let x be the frequency of homolog segregation to opposite poles, and $1 - x$ the frequency of missegregation. The frequencies of zero, one, two, and three homolog segregation errors can be calculated as shown in the second column of Table 2. The frequencies of one and two errors occurring must include an additional factor of three since one error can occur with any one of the three chromosomes, and two errors can occur with any of the three possible combinations of two chromosomes. Therefore, one and two errors can occur three times as often as zero and three errors.

As well as the number of errors, the exact nature of the meiotic products that result from missegregation will depend on which of the two MI poles the homologs move to. That is, either pole A or pole B can receive a pair of homologs (designated I and I′, II and II′, and III and III′). Therefore, a factor of eight is introduced (2^3 is the no. of poles$^{\text{no. of chromosomes}}$)

TABLE 2

Frequency of meiotic products resulting from MI homolog missegregation

No. of missegregated homolog pairs	Frequency of occurrence	(Possible groupings) × (frequency of occurrence)	Frequency of meiotic products
0	x^3	$8\ x^3$	Haploids[a]: $8\ x^3$
1	$3x^2(1-x)$	$24\ x^2(1-x)$	ChrIII disomes[a]: $4\ x^2(1-x)$
			Dead[b]: $20\ x^2(1-x)$
2	$3x(1-x)^2$	$24\ x(1-x)^2$	Dead[c]: $24\ x(1-x)^2$
3	$(1-x)^3$	$8(1-x)^3$	Diploid[a]: $1\ (1-x)^3$
			Dead[c]: $7\ (1-x)^3$

See MATERIALS AND METHODS for explanation. x is the frequency of proper chromosome segregation.
[a] Viable.
[b] Nullosomic for any chromosome or disomic for ChrI or ChrII.
[c] Multiply nullosomic or disomic.

for all the classes, zero, one, two, and three errors. For instance, in the class with three errors, pole A can receive any of the following eight groupings of three chromosomes: (1) none; (2) I, I′; (3) II, II′; (4) III, III′; (5) I, I′ and II, II′; (6) I, I′ and III, III′; (7) II, II′ and III, III′; and (8) I, I′ and II, II′ and III, III′. The chromosomes that occupy pole B are determined by those at A; thus, there are eight ways to accomplish three errors. The same is true of the class with zero errors and all the subclasses of one and two errors (one error involving ChrI, one error involving ChrII, etc.). The importance of the random assortment of errors to either of two poles is underscored in the example above with three errors. One of the above groupings of chromosomes [(8) I, I′ and II, II′ and III, III′] results in two diploid spores after MII, while all the other groupings are nullosomic for at least one chromosome and will therefore give rise to two inviable spores after MII. This final step in the calculation of the frequency of zero, one, two, and three errors occurring is shown in the third column of Table 2. The fourth column of Table 2 indicates the nature of the meiotic products that result. Using column four of Table 2, we have graphed the frequency of viable spores, heterozygous diploids, and ChrIII disomes expected at reductional segregation rates of 50% (random; $x = 0.5$) to 100% ($x = 1.0$; Figure 3).

Detecting aneuploid meiotic products: The frequency of aneuploid meiotic products was determined by random spore analysis. While random spore analysis cannot differentiate between homolog missegregation and PSSC, we chose this method because the abnormal spore morphology of *rec12* mutants (see Figure 4) could bias the selection of asci for dissection and analysis of tetrads. Additionally, differential plating of random spores allowed us to maintain selection for the unstable ChrIII disomes.

Spores were liberated from asci, and vegetative cells were killed, by treatment with glusulase and ethanol (PONTICELLI and SMITH 1989). Spore suspensions were plated on supplemented EMM2 to detect I_2-staining spore colonies (*mat1-P/mat1-M* heterozygous diploids; BRESCH *et al.* 1968) and on YEA + 4S + 100 μg/ml guanine (YEAG), which inhibits uptake of adenine (CUMMINS and MITCHISON 1967), to detect adenine-prototrophic spore colonies. Both *ade6-M210/ade6-M216* heterozygous diploids and heterozygous ChrIII disomes grow on YEAG. Heterozygous diploids form large colonies after 3 days, while heterozygous ChrIII disomes form small colonies only after 4 or more days. Because heterozygous diploids were detected in the Spo^+ assay above, only the small colonies (ChrIII disomes) were counted on YEAG. Spore suspensions were plated on YEA + 4S to determine the total number of viable spores and on YEA + 5S to determine the frequency of diploid spore colonies. The cells from random spore colonies were examined microscopically. Ploidy was initially determined by cell size and confirmed by flow cytometry (data not shown). The phenotypes of diploid spore colonies were checked by streaking to YEA + 4S and, after 3 or 4 days growth, replica plating to supplemented EMM2 ± 100 μg/ml lysine and YEA + 5S with or without 100 μg/ml cycloheximide. The phenotypes of the spores dissected from *rec12*Δ dyads were determined in a similar manner. Spore viability was determined as follows: asci were suspended in sterile water and spores were liberated from asci, and vegetative cells were killed by treatment with glusulase and ethanol (PONTICELLI and SMITH 1989). The spore suspension was examined by light microscopy to determine the number of total spores and dilutions were plated to YEA + 4S to determine the number of viable spores.

Microscopy: Approximately 10^7 cells of each parent strain were mated on supplemented SPA and, after 2–3 days, the matings were collected and suspended in 500 μl of sterile water. A 100-μl aliquot of this suspension was added to 1 ml of prechilled −20° methanol. After 20–30 min at −20°, the cells were pelleted, washed once in water, resuspended in 200 μl PEMS (1.2 M sorbitol, 100 mM Pipes pH 6.9, 1 mM EGTA, 1 mM $MgSO_4$), and stored at 4°. To stain chromatin, 20 μl of the fixed ascus suspension was added to 1 ml of PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 pH 7.4) and pelleted. The pellet was resuspended in ~50 μl of PBS and 10 μl was placed on a microscope slide. The slide was heated for 1–2 min at 70° to provide a monolayer of cells adhered to the slide. Mounting medium (50% glycerol, 1 mg/ml *p*-phenylenediamine, 1 μg/ml of the fluorescent DNA-dye Hoechst 33342) was used. The same procedure was used for *mes1*Δ zygotes except in this case the cells were not fixed prior to viewing.

For detection of GFP13-LacI12-NLS-marked chromosomes, live cells were observed. Cells were mated on supplemented SPA and, after 2–3 days, zygotes were examined. By this time ~95% of zygotes had undergone MI. Fluorescence microscopy was performed on a Nikon Eclipse 600 microscope using a Nikon 60× 1.4 NA Plan Apo objective (Nikon, Melville, NY). Images were captured on a CoolSNAP CCD camera (Roper Scientific, San Diego).

Recombination frequencies: The appropriate strains were crossed on supplemented SPA and, after 2–4 days, spores free of viable vegetative cells were prepared by treatment with glusulase and ethanol (PONTICELLI and SMITH 1989). Spore suspensions were plated on YEA + 5S and, after 3–5 days,

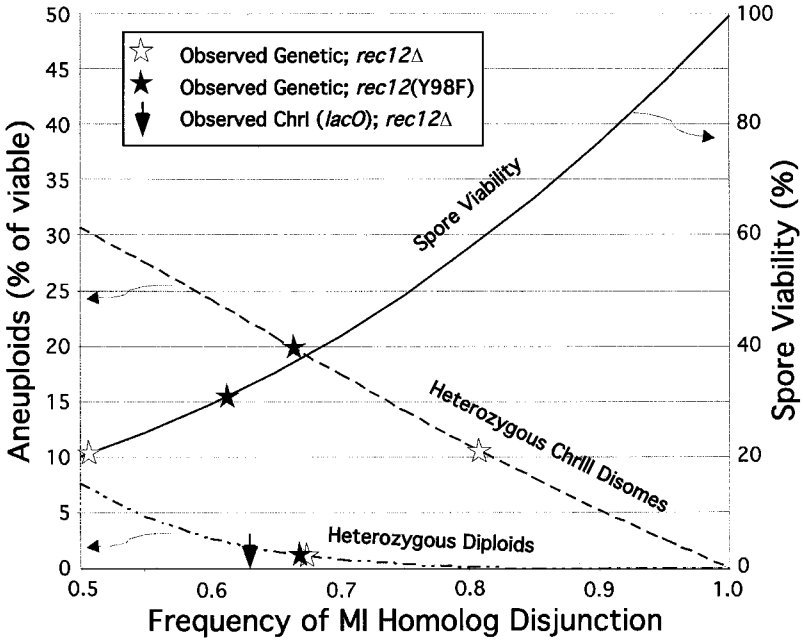


FIGURE 3.—Expected frequency of *rec12* meiotic products characteristic of MI homolog missegregation at segregation frequencies of 50–100%. The derivation of the formulas used to construct the graphs is described in MATERIALS AND METHODS. The observed frequencies of *rec12* meiotic products characteristic of MI homolog missegregation (Table 5) have been plotted on the appropriate expectation curve and are indicated by open stars for *rec12*Δ and solid stars for *rec12*(Y98F). Observed spore viabilities have been normalized to wild type. The vertical arrow indicates the frequency of proper segregation by ChrI as observed cytologically (Table 7).

colonies were toothpicked to grids on YEA + 5S. After growth overnight, the segregants were replicated to the appropriate test media: EMM2 ± adenine for *ade4-31*, ± leucine for *leu2-120*, and ± uracil for *ura1-61* and *spc1::ura4*$^+$. All of the *rec12*Δ mutant recombinants were purified and retested to verify the scoring.

RESULTS

Expected frequency of aneuploid meiotic products in segregation-defective mutants: Missegregation of homologous chromosomes at MI should result in spore inviability and heterozygous diploid and aneuploid spores (Figure 2). If homologs segregate randomly at MI in the absence of recombination, the spores of a *rec* mutant meiosis should yield a characteristic frequency of viable haploids, heterozygous diploids, and heterozygous ChrIII disomes, the only aneuploids that have been propagated in *S. pombe* (NIWA and YANAGIDA 1985; MOLNAR and SIPICZKI 1993). As a framework for interpretation of our segregation data, we calculated the frequency of viable spores, heterozygous diploids, and ChrIII disomes expected at reductional segregation rates of 50% (random) to 100% (Figure 3; see MATERIALS AND METHODS for derivation).

Several assumptions were made to simplify the calculations: (1) The only type of segregation error is MI homolog missegregation, (2) missegregation of homologs does not preclude microscopically visible spore formation, (3) all three homologs segregate with equal efficiency, and (4) ChrIII disomes are viable, while ChrI and ChrII aneuploids are inviable. The validity of these assumptions and the effect of deviations from them are considered in the DISCUSSION. Importantly, significant deviation from any of these assumptions should be obvious when the results of genetic and physical analysis of segregation are compared.

Spore morphology and DNA staining in recombinationless meiosis: To begin to assess meiotic segregation in the absence of recombination, we characterized the morphology and DNA content of Hoechst 33342-stained *rec*$^+$ and *rec12*Δ asci using differential interference contrast and fluorescence microscopy. The four spores in *rec*$^+$ asci (tetrads) all appeared to be the same size and contained apparently equivalent amounts of DNA (Figure 4A). Many asci with morphologies not seen in wild type were frequently observed in *rec12*Δ crosses. Dyads were frequently formed in *rec12*Δ meiosis (Figure 4, B and C; Table 3). The spores in the dyad asci frequently contained two Hoechst 33342-staining bodies (Figure 4C). Three-spored asci containing one large spore, with two Hoechst 33342-staining bodies, and two smaller spores were also observed (Figure 4F). Other less common classes of aberrant asci were also observed in *rec12*Δ crosses (data not shown). The size of spores and the distribution of DNA were often uneven in both dyad and tetrad *rec12*Δ asci (Figure 4, B–F). This suggests that chromosomes missegregated at MI and/or MII in *rec12*Δ mutants.

To eliminate the contribution of any MII errors in DNA distribution, the *mes1::LEU2* (*mes1*Δ) mutation, which blocks MII (SHIMODA *et al.* 1985; KISHIDA *et al.* 1994; Table 7), was used. Both *rec*$^+$ and *rec12*Δ strains containing the *mes1*Δ mutation were induced to mate and to undergo MI. Cells were examined as above. DNA was evenly distributed between the two MI poles in *rec*$^+$ *mes1*Δ (Figure 4G) zygotes. Uneven distribution of DNA

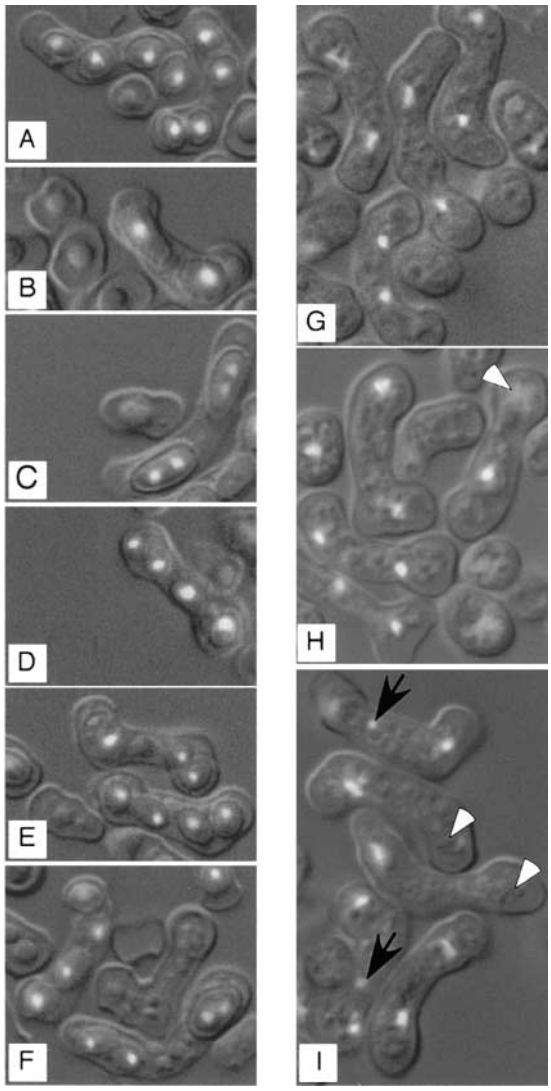


FIGURE 4.—Ascus morphology and DNA distribution in *rec* mutants indicates the occurrence of chromosome missegregation and frequent single-division meiosis. Photomicrographs are shown combining both visible light and fluorescence images of Hoechst 33342-stained asci (A–F) or zygotes (G–I). Hoechst staining is distributed evenly between the spores in *rec*$^+$ asci (A). (B and C) *rec12*Δ dyad asci containing one or two nuclei per spore, respectively. (D) *rec12*Δ ascus with unevenly distributed DNA. (E and F) Additional *rec12*Δ asci, including one with four spores containing apparently equivalent amounts of DNA. (G) *rec*$^+$ *mes1*Δ zygotes. Hoechst 33342 staining is distributed evenly between the MI poles. (H and I) *rec12*Δ *mes1*Δ zygotes. Black arrows indicate lagging chromosomes. White arrowheads indicate zygotes with one pole apparently devoid of DNA.

between the two MI poles in *rec12*Δ *mes1*Δ zygotes (Figure 4, H and I) indicates that chromosomes at least occasionally missegregate at MI. Additionally, lagging chromosomes were occasionally observed in *rec12*Δ *mes1*Δ zygotes (Figure 4I). The abnormalities we observed, including dyad spore formation, uneven distribution of DNA, and lagging chromosomes in *rec12*Δ meioses, are similar to those of *rec7*, *rec14*, and *rec15* mutants (MOLNAR *et al.* 2001a,b).

The dyads observed in *rec12*Δ meiosis frequently contain homozygous diploid spores, the products of proper reductional division: A striking feature of *rec12* meioses was the high frequency of dyad asci (Figure 4, B and C; Table 3). Analysis of random spore colonies indicated that *rec12*Δ meioses yielded a high frequency of diploid spores. Strains marked on all three chromosomes were used to determine the types of meiotic errors that gave rise to these diploids. Chromosomes I, II, and III were marked with *lys1*$^+$ *cyh1-101/lys1-37 cyh1*$^+$, *mat1-P/mat1-M*, and *ade6-M210/ade6-M216* alleles, respectively. In wild-type strains the *lys1* and *ade6* alleles are centromere linked (*lys1*, 4 cM; *ade6*, 13 cM; KOHLI *et al.* 1977). In *rec12*Δ mutants the *mat1* alleles were also centromere linked (2 cM; data not shown).

Both MI and MII segregation errors can give rise to diploid spore colonies. Centromere-linked markers will be heterozygous if the diploids are due to MI errors or homozygous if the diploids are due to MII errors (Figure 2). To determine which type of error occurs in *rec12*Δ meioses, the marked strains were sporulated and random spores plated on rich medium. Cells from the resulting colonies were examined. Diploid cells were easily distinguished by their size: haploid and diploid cells are ~14 and ~22 μm long, respectively, when cell division occurs (SVEICZER *et al.* 1996). Ploidy was confirmed by flow cytometry (data not shown). While only 3% of the *rec*$^+$ spore colonies were diploid, >25% of *rec12*Δ spore colonies were diploid (Table 3). The marker phenotypes were determined and indicated that the *rec12*Δ diploid spores were predominantly (88%) homozygous for the centromere markers (Table 3). This indicates that these *rec12*Δ diploid spores were the result of a single reductional (MI) division. A substantial portion of the observed heterozygosity was in mixed segregation diploids. These may have resulted from a single reductional division, during which homologs missegregated, followed by mitotic chromosome loss.

Dissection of the *rec12*Δ dyad asci was performed to determine whether they were the source of the *rec12*Δ diploid spores. Of the 69 dyads dissected, 37 contained two viable spores (Table 4). The vast majority (34/37) of the two-spore viable dyads contained two homozygous diploid spores. Furthermore, 10 of 17 one-spore viable dyads contained a homozygous diploid spore, suggesting that chromosome loss occurred either during meiosis or after germination of an aneuploid product of MI missegregation. This indicates that the *rec12*Δ dyad asci were at least a major source of the *rec12*Δ diploid spores.

The dyad dissections also suggest that MI homolog segregation was not random in the *rec12*Δ meioses. Random segregation is expected to result in proper segregation of all three chromosomes in only 12.5% of meioses ($0.5^3 = 0.125$). However, 49% (34/69) of *rec12*Δ dyads

TABLE 3

***rec12* mutant meiosis frequently yields dyad asci and homozygous diploid spores, the products of proper reductional division**

	rec$^+$ (GP2659)	*rec12*Δ (GP2657)	*rec8*Δ (GP2656)	*rec12*Δ *rec8*Δ (GP2658)
Diploid spores/total random spores[a]	3% (3/108)	28% (19/68)	10% (7/68)	9% (6/68)
No. of homozygous chromosome pairs[b] (MII error)	8	50	6	6
No. of heterozygous chromosome pairs[b] (MI error)	1	7	15	12
Dyad asci/total asci[c]	0.4%	30%	0.5%	1.2%

[a] The cells from random spore colonies were examined microscopically and the ploidy was determined.
[b] Each chromosome was marked genetically. The diploid spore colonies were checked phenotypically (see MATERIALS AND METHODS) to determine whether each pair of chromosomes was homozygous or heterozygous.
[c] Sporulated cultures were examined microscopically. More than 200 asci were counted for each strain.

contained two homozygous diploid spores and were therefore the result of a single MI division in which all homologs segregate properly (Table 4). Together with the high spore viability of *rec12*Δ dyads (66%; Table 4), this indicates that MI homolog segregation is not random in *rec12*Δ meioses, at least not for those that form dyads.

The frequency of meiotic products characteristic of MI missegregation indicates *rec12*Δ mutants segregate homologs nonrandomly: Although analysis of *rec12*Δ diploid spore colonies indicated that segregation was nonrandom, several one-spore viable dyads contained aneuploid spores indicative of MI homolog missegregation. As discussed above (see Figure 3), missegregation of homologs at MI should result in a predictable level of spore inviability and in the formation of heterozygous diploids and ChrIII disomic spores. Values for these parameters were determined for both *rec12*Δ and the presumptive catalytically inactive mutant *rec12*(Y98F) to begin to assess the degree of MI homolog missegregation in *rec12* meiosis. The validity of this approach depends on the unbiased encapsulation of meiotic products into spores. Although a few zygotes did not form visible spores (data not shown), in the DISCUSSION we argue, on the basis of the entirety of our data, that encapsulation is essentially unbiased.

Random segregation of the three homologs at MI is predicted to decrease spore viability to 20.3% (Figure 3). As the abnormal spore morphology of *rec12* mutants could bias the selection of asci for dissection, we chose to assay viability by determining the fraction of random spores, enumerated by microscopy, that formed visible colonies. Although there was significant variability from experiment to experiment, *rec*$^+$ meioses resulted in ~70% spore viability, most likely due to difficulty in scoring spores microscopically and/or to excess treatment with glusulase. However, as expected, *rec*$^+$ spore viability [69.4 ± 8.6% (mean ± SEM)] was significantly

TABLE 4

Dissection indicates that *rec12* mutant dyads contain predominantly homozygous diploid spores, the products of proper reductional division

	No. of dyads with *x* viable spores		
Spore type	*x* = 2	*x* = 1	*x* = 0
Homozygous diploid	34	10	—
Haploid	0	4	—
Other	2 (2*n* and 1*n*)[a]	2 (2*n* + 2 chrI)[c]	—
	1 (2*n* and 2*n*)[b]	1 (2*n* + 2 chrIII)[d]	
Total dyads	37	17	15

Dyad asci from strain GP2657 were dissected and the phenotype of each viable spore was determined.
[a] These two dyads each contained one homozygous diploid and one haploid spore.
[b] This dyad contained two mixed segregation spores.
[c] These two dyads each contained one aneuploid spore that was homozygous for chromosomes II and III and contained three or four copies of chromosome I.
[d] This dyad contained one aneuploid spore that was homozygous for chromosomes I and II and contained three or four copies of chromosome III.

TABLE 5

Random spore analysis: fewer than expected heterozygous spores from MI missegregation indicate that *rec12* mutants segregate homologs nonrandomly

Parental genotype	Strains	Heterozygous ChrII[a] (% of viable spores)	Heterozygous ChrIII[b] (% of viable spores)	Spore viability[c]
rec$^+$	(GP3714 × GP3716)	0.6 ± 0.1	<0.1 ± 0.01	69.4 ± 8.6%
*rec12*Δ	(GP3715 × GP3717)	1.3 ± 0.2	10.5 ± 1.2[d]	14.5 ± 4.2%
rec12 (Y98F)	(GP3684 × GP3712)	1.4 ± 0.2	19.3 ± 2.0[d]	21.8 ± 1.7%
rec12 (Y98F)/*rec12*Δ	(GP3684 × GP3715) (GP3712 × GP3717)	1.3 ± 0.1	15.0 ± 0.9[e]	17.1 ± 2.2%
*rec8*Δ	(GP3764 × GP3765)	10.3 ± 0.6	21.7 ± 2.0[f]	14.9 ± 1.0%
*rec12*Δ *rec8*Δ	(GP3766 × GP3767)	9.3 ± 0.4	30.0 ± 1.3[f]	16.5 ± 1.3%

Values are given as mean ± the standard error of the mean ($n = 6$).

[a] Spores were plated on EMM2 + supplements to assay ChrII heterozygosity. Colonies were stained with I_2 to determine the frequency of Spo$^+$ (*mat1-P/mat1-M* heterozygous) spores.

[b] Spores were plated on YEAG to assay ChrIII heterozygosity. Ade$^+$ colonies were confirmed to be heterozygous *ade6-M210/ade6-M216* by the ability to segregate *ade6* mutant cells.

[c] Spores were plated on YEA + 4S to assay viability. The number of total spores was determined microscopically (see MATERIALS AND METHODS).

[d] These values are significantly different on the basis of two-tailed *t*-tests of independent samples ($P < 0.005$).

[e] On the basis of two-tailed *t*-tests of independent samples, this value is significantly different from the corresponding value for *rec12*Δ meiosis ($P < 0.025$), but is not significantly different from the value for *rec12* (Y98F) meiosis ($0.1 > P > 0.05$).

[f] These values are significantly different on the basis of two-tailed *t*-tests of independent samples ($P < 0.01$).

higher than that of the *rec12* mutants. Spore viability in *rec12*Δ and *rec12*(Y98F) meioses was only 14.5 ± 4.2% and 21.8 ± 1.7%, respectively (Table 5).

Random segregation at MI also predicts that 7.7 and 30.7% of total viable spores would be heterozygous diploids and ChrIII disomes, respectively (Figure 3). To reduce bias, as with spore viability, we chose to assay heterozygous diploids and ChrIII disomes among random spores rather than by tetrad dissection. Heterozygous diploid spores, which have one copy each of the *mat1* alleles *P* and *M*, are sporulation proficient (Spo$^+$). Only ~1.3 ± 0.2% and 1.4 ± 0.2% of the viable spores produced in *rec12*Δ and *rec12*(Y98F) meioses, respectively, were Spo$^+$ (Table 5). This is not greatly different from the 0.6 ± 0.1% Spo$^+$ spores observed in *rec*$^+$ crosses (Table 5). The intragenic complementation displayed by the *ade6* alleles *M210* and *M216* allows missegregation of ChrIII to be assayed by formation of Ade$^+$ (white) colonies. While 0.1 ± 0.01% of *rec*$^+$ viable meiotic products were Ade$^+$, 10.5 ± 1.2% of *rec12*Δ and 19.3 ± 2.0% of *rec12*(Y98F) viable meiotic products were heterozygous ChrIII disomes (Table 5). Both the *rec12*Δ and *rec12*(Y98F) heterozygous ChrIII disome frequencies were significantly <31%, the frequency predicted for random segregation at MI ($\chi^2 = 137$, $P \ll 0.001$ and $\chi^2 = 80$, $P \ll 0.001$, respectively). These results are all consistent with the hypothesis that, in the absence of meiotic recombination, chromosomes segregate reductionally more frequently than random segregation predicts.

Visualization of ChrI indicates that *rec12*Δ mutants segregate homologs nonrandomly: Genetic analysis of segregation is limited to viable meiotic products. The results above, which are consistent with nonrandom segregation of homologs at MI, could also be explained if the products of properly executed meiotic divisions are preferentially encapsulated into spores. To overcome this limitation, we used strains whose ChrI centromeres were marked with a tandem array of *lacO* DNA (NABESHIMA *et al.* 1998). This array can be visualized by fluorescence microscopic observation of GFP-LacI-NLS fusion proteins that bind it. The *mes1*Δ mutation was again used to simplify the analysis by blocking MII and thereby eliminating the contribution of any MII errors. None of the results described below were dependent on the *mes1*Δ mutation, as in all cases tested similar results were obtained in a *mes1*$^+$ background (data not shown).

First, we determined whether sister chromatids separate prematurely prior to MI in *rec12*Δ mutant meioses (Figure 5A and Table 6). In crosses heterozygous for the *lacO* array, if the first division is reductional, both sister chromatids containing the array will segregate to one pole. One *lacO*-containing sister chromatid at each pole indicates either an equational first division or a PSSC. In *rec*$^+$ *mes1*Δ crosses 98% of cells had a GFP signal at only one pole (Table 6). Similarly, in 96% of *rec12*Δ *mes1*Δ meioses the *lacO* arrays segregated to only one pole (Table 6). These data establish that sister chromatids do not frequently separate prior to MI in *rec12*Δ mutants.

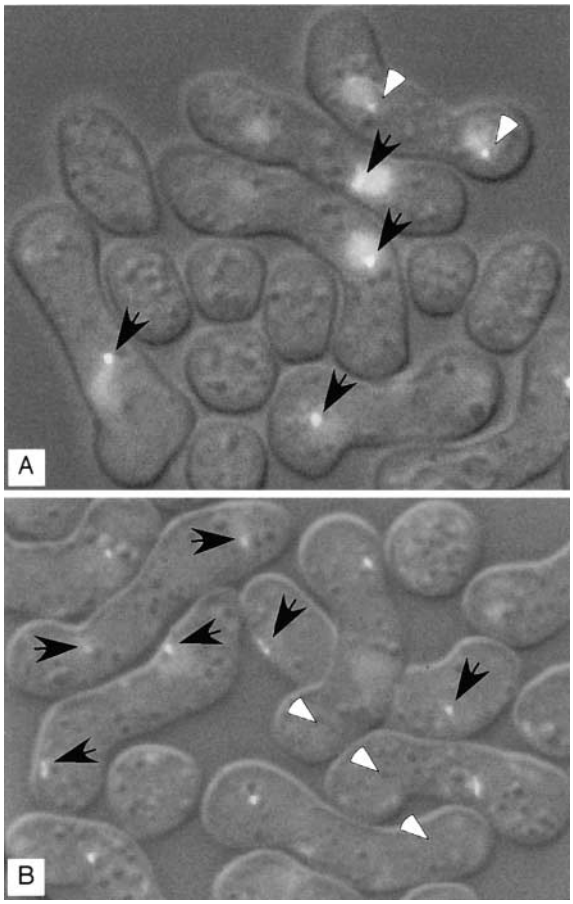


FIGURE 5.—Chromosome I homolog segregation is nonrandom in *rec12* mutant meiosis. A *lacO* array integrated near the centromere of chromosome I is detected by a GFP-LacI fusion protein in *mes1*Δ meioses. Photographs are of *rec12*Δ *mes1*Δ strains with (A) heterozygous *lacO* arrays and (B) homozygous *lacO* arrays. In A, black arrows indicate proper segregation of sister chromatids to one pole and white arrowheads indicate a single sister-chromatid missegregation event. In B, black arrows indicate proper segregation of homologs to opposite poles in three zygotes and white arrowheads indicate poles without a marked ChrI homolog indicative of zygotes that have undergone homolog missegregation.

To segregate properly at MII, sister chromatids must maintain cohesion at the centromere until the metaphase-to-anaphase transition of MII. Even if MI segregation is reductional, loss of cohesion prior to MII would allow the *lacO* arrays to diffuse away from each other after MI (BERNARD *et al.* 2001), resulting in two *lacO* signals at the same pole. Two distinct *lacO* signals were seen at the same pole 17% of the time in *rec*$^+$ *mes1*Δ (Table 6) and 11% of the time in *rec12*Δ *mes1*Δ (Table 6). The relatively high number of zygotes with two distinct signals at one pole was likely due to the duration of the *mes1*Δ arrest. However, these data do indicate that sister-chromatid cohesion is maintained after MI in *rec12*Δ meioses as well as it is in *rec*$^+$ meioses.

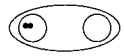
To determine the frequency of proper homolog segregation at MI, *mes1*Δ crosses homozygous for the *lacO* array were performed and zygotes were examined by fluorescence microscopy after 2–3 days (Figure 5B). In these crosses proper segregation will result in one *lacO*-containing homolog at each pole. In *rec*$^+$ *mes1*Δ crosses the *lacO* arrays segregated to opposite poles in 99% of the meioses (Table 7). In *rec12*Δ *mes1*Δ crosses the *lacO* arrays segregated to opposite poles in 63% of the meioses (Table 7). The frequency of proper segregation in *rec12*Δ (63%) is significantly higher than the expected frequency of 50% ($\chi^2 = 21$: $P < 0.001$).

Spontaneous, Rec12-independent recombination is not sufficient to account for the observed level of proper homolog segregation: The degree of nonrandom segregation observed using the *lacO* array on ChrI in *rec12*Δ mutants could be explained if Rec12-independent recombination resulted in one or more crossovers on ChrI in 26% of meioses. This is equivalent to ChrI having a genetic length of 13 cM. To address this possibility, we performed random spore analysis of crosses in which we could measure recombination between *spc1* (SHIOZAKI and RUSSELL 1995) and *ade4* near the ends of ChrI. These markers are separated by 5.1 Mb (WOOD *et al.* 2002), thus covering 89% of ChrI (5.7 Mb). Although *spc1* has not been placed on the genetic map, its physical proximity to *cdc12* (WOOD *et al.* 2002) indicates that the genetic distance separating *spc1* and *ade4* in a *rec*$^+$ meiosis would be ~900 cM (MUNZ *et al.* 1989). As expected, *spc1* and *ade4* were unlinked in *rec*$^+$ crosses (Table 8). In *rec12*Δ crosses the recombination frequency between *spc1* and *ade4* was 2.1% (= 2.2 cM, Table 8). Similar results were obtained with *ura1* and *leu2* markers, which cover 63% of ChrI (Table 8). Extrapolating from the *spc1* to *ade4* data gives a genetic length of 2.5 cM for ChrI (2.2 cM/0.89). This is significantly lower ($\chi^2 = 61$: $P \ll 0.001$) than the 13 cM required to explain the observed degree of nonrandom segregation as being directed by Rec12-independent recombination.

The absence of recombination, rather than of some other function of Rec12, results in the observed segregation phenotypes: It has been reported that the Rec12 homolog, Spo11, has roles in meiotic progression other than DSB formation in *S. cerevisiae* and Coprinus (CHA *et al.* 2000; MERINO *et al.* 2000). Additionally, localization of Spo11 along synapsed chromosomes in mouse (ROMANIENKO and CAMERINI-OTERO 2000) suggests a role in stabilizing homolog interactions. This raises an important question: Are the segregation phenotypes that we observe in *rec12*Δ strains due solely to the absence of recombination? The segregation phenotype is not specific to *rec12*Δ as all the strong *rec* mutants whose only known meiotic defect is in recombination, *rec6*, *rec7*, *rec12*, *rec14*, and *rec15* (LIN *et al.* 1992; LIN and SMITH 1994, 1995; EVANS *et al.* 1997), displayed an increased frequency of dyad asci and diploid spores and a low level of Spo$^+$ diploid spores (Table 9; DEVEAUX and SMITH 1994; MOLNAR *et al.* 2001b). This suggests

TABLE 6

Sister chromatid cohesion is unaffected in *rec12* mutants

	Sister chromatids at MI		
	Proper segregation		
Parental genotypes	With cohesion (%) 	Without cohesion (%) 	Missegregation (%) 
rec$^+$ (GP3757 × GP3761)	81	17	2
*rec12*Δ (GP3758 × GP3762)	86	11	4

Crosses heterozygous for the *lacO* array were performed with *mes1*Δ parental strains that were either *rec*$^+$ or *rec12*Δ. More than 200 zygotes/cross were examined by fluorescence microscopy after 2–3 days on sporulation medium and were scored for the number and location of GFP-LacI-NLS signals.

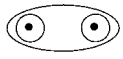
that these gene products act together, perhaps in a complex, or that the absence of recombination *per se* results in the common phenotype.

Tyrosine-135 of Spo11 is thought to be directly involved in creating meiotic DNA double-strand breaks and presumably forms a phosphodiester link between Spo11 and DNA by a topoisomerase-like mechanism (Bergerat *et al.* 1997; Keeney *et al.* 1997). Spo11 (Y135F) mutant protein is not capable of promoting meiotic recombination yet appears to perform Spo11's additional roles in meiotic progression (Cha *et al.* 2000). The homologous tyrosine is replaced by a phenylalanine in Rec12(Y98F), which is encoded by the *rec12-164* allele, differing from wild type only by the absence of the presumptive reactive oxygen atom. The *rec12-164* allele is strongly recombination deficient (Cervantes *et al.* 2000) and, like the other strong *rec* mutants (Table 9), displayed a low level of Spo$^+$ diploid spores and an increased frequency of dyad asci and diploid spores (Table 5; data not shown). The *rec12-164* allele (Rec12-Y98F) may result in production of an unstable protein or of a protein that does not properly form a complex. We think, however, that these data are most consistent with the notion that the absence of recombination, rather than of some other function of Rec12, results in the observed segregation phenotypes.

Rec8 is required for the segregation phenotypes displayed by *rec12*Δ mutants: To determine whether the Rec8 cohesin protein is required for nonrandom segregation, *rec8::ura4*$^+$ (*rec8*Δ) single-mutant and *rec12*Δ *rec8*Δ double-mutant strains were investigated. These strains were marked on all three chromosomes to determine the frequency of meiotic products characteristic of MI missegregation. The frequency of heterozygous diploid (Spo$^+$) spores from both *rec8*Δ and *rec12*Δ *rec8*Δ mutant crosses (Table 5; 10.3 ± 0.6% and 9.3 ± 0.4%, respectively) was consistent with random segregation, which predicts 7.7% heterozygous diploid spores (Figure 3). Interestingly, *rec12*Δ *rec8*Δ mutant crosses produced many fewer dyad asci than *rec12*Δ mutant crosses and about the same as *rec*$^+$ (Table 3). While these data indicate that *rec8* is epistatic to *rec12* with regard to meiotic segregation, the frequency of meiotic products disomic for ChrIII (Ade$^+$) suggests a more complicated relationship. Random segregation predicts that 30.7% of the viable meiotic products would be disomic for ChrIII

TABLE 7

Cytological analysis: MI homolog segregation is nonrandom in *rec12* mutants

	Homologous chromosome at MI	
	Segregation (%)	Missegregation (%)
Parental genotype		
rec$^+$ (GP3757 × GP3759)	99 (178/179)[a]	1
*rec12*Δ (GP3758 × GP3760)	63 (383/607)[a]	37

Crosses homozygous for the *lacO* array were performed with *mes1*Δ parental strains that were either *rec*$^+$ or *rec12*Δ. Zygotes were examined by fluorescence microscopy after 2–3 days on sporulation medium and were scored for the number and location of GFP-LacI-NLS signals.

[a] Segregation data are from three experiments. The raw data from these three experiments, expressed as cells with proper segregation/total cells, were 51/51, 20/20, and 107/108 for *rec*$^+$ and 123/199, 64/104, and 196/304 for *rec12*Δ.

TABLE 8

Rec12-independent recombination is not sufficient to explain the observed nonrandom segregation

Parental genotype	Intervals	
	spc1-ade4 (5.1 Mb ChrI)	*ura1-leu2* (3.6 Mb ChrI)
rec$^+$[a]	~900 cM[c] (144/291)	~700 cM[c] (111/191)
*rec12*Δ[b]	2.2 cM[d] (20/948)	1.4 cM[d] (10/709)

[a] *rec*$^+$ strains GP3533 and GP3535 were crossed to assay the *spc1*-to-*ade4* interval, and GP3570 and GP3572 were crossed to assay the *ura1*-to-*leu2* interval.

[b] *rec12*Δ strains GP3534 and GP3536 were crossed to assay the *spc1*-to-*ade4* interval, and GP3571 and GP3573 were crossed to assay the *ura1*-to-*leu2* interval.

[c] The *rec*$^+$ genetic distances were estimated on the basis of the genetic map of Munz *et al.* (1989). The number of recombinants/total spore colonies is given in parentheses.

[d] The *rec12*Δ recombinant frequencies were converted to genetic distance using Haldane's formula, cM = $-50 \ln(1 - 2R)$, where R is the recombinant frequency. The number of recombinants/total spore colonies is given in parentheses.

(Figure 3); however, we observed 21.7 ± 2.0% in *rec8*Δ meioses and 30.0 ± 1.3% in *rec12*Δ *rec8*Δ meioses (Table 5). This difference between *rec8*Δ and *rec12*Δ *rec8*Δ is statistically significant ($P < 0.01$; Table 5) and indicates that Rec12 and Rec8 independently promote homolog segregation.

DISCUSSION

In most organisms, reciprocal recombination in conjunction with SCC provides the connection between homologous chromosomes required for proper MI segregation. While this appears to be generally true, mechanisms exist in many organisms that promote the proper segregation of achiasmate chromosomes. In Drosophila, which possesses chromosomes that never recombine, these mechanism are nearly 100% effective (reviewed in Hawley and Theurkauf 1993). *S. cerevisiae*, in which even the shortest chromosomes only rarely fail to undergo at least one reciprocal recombination event (Kaback *et al.* 1989), can promote the proper segregation of achiasmate artificial chromosomes (Dawson *et al.* 1986) as well as two monosomic nonhomologous chromosomes (Guacci and Kaback 1991). Here, we first discuss the gross cytology of the meiotic divisions and spore formation in *S. pombe* recombination-deficient mutants. We then provide evidence that in the absence of meiotic recombination homologous chromosomes segregate to opposite poles more frequently than predicted by random segregation.

The dyads produced in *rec12*Δ meioses frequently contain two homozygous diploid spores: We frequently observed that *rec12*Δ meioses produced two-spored (dyad) asci (Figure 4, B and C). The spores in these dyads frequently contained two nuclei (Figure 4C). After submission of this article, the presence of dyad asci containing spores with two nuclei in *rec12* mutants was also reported by Sharif *et al.* (2002). Additionally, *rec12*Δ dyads frequently contain two homozygous diploid spores (Table 4); these are clearly the products of

TABLE 9

Other strong *rec* mutants share segregation phenotypes with *rec12* mutants

Genotype	Dyad asci (%)[a]	Diploid spores/ total tested[a]	ChrII homozygous/ diploids[a]	Spo$^+$ (% of total tested)
rec$^+$ (GP720 × GP2183)	0.4	0/50	NA	1.2[b]
rec6 (GP1147 × GP1253)	21	7/54	7/7	1.3[c]
rec7 (GP821 × GP985)	18	5/57	3/5	1.0[d]
rec12 (GP1456 × GP1459)	21	5/46	5/5	0.8[c]
rec14 (GP1994 × GP2004)	23	8/30	7/8	2.7[e]
rec15 (GP1688 × GP1825)	13	10/30	10/10	0.8[c]

[a] These data are from one experiment. More than 300 asci were scored for dyad frequency.

[b] This datum is the mean from six experiments, five of which used parents other than GP720 and GP2183.

[c] This datum is the mean from two experiments.

[d] This datum is the mean from six experiments, five of which used parents other than GP821 and GP985.

[e] This datum is the mean from four experiments, three of which used parents other than GP1994 and GP2004.

a single reductional division. Meioses in *rec7*, *rec14*, and *rec15* mutants also produce dyad asci that contain spores with two nuclei (Molnar *et al.* 2001a). Like *rec12*Δ dyads, *rec7* dyads frequently contain homozygous diploid spores (Molnar *et al.* 2001b).

The *rec12*Δ dyads are reminiscent of those formed by several cell division cycle (*cdc*) mutants. Cells with mutations in the mitotic initiation genes *cdc2*, *cdc25*, or *cdc13* are defective in the initiation or completion of MII, frequently undergoing a single reductional meiotic division and forming dyad asci when sporulated at semipermissive temperature (Nakaseko *et al.* 1984; Niwa and Yanagida 1988; Grallert and Sipiczki 1991). Like *rec12*Δ dyads (Figure 4C), *cdc25* dyads often contain spores that enclose two nuclei (Grallert and Sipiczki 1989). One difference between the dyads formed in *rec12*Δ mutants and those formed in *cdc2* mutants is that *rec12*Δ dyad formation requires the meiosis-specific sister-chromatid cohesin Rec8 (Table 3) while *cdc2* dyad formation does not (Watanabe and Nurse 1999). There are at least two possible interpretations of the dependence of dyad formation on Rec8.

First, the absence of tension in *rec12* meiosis may activate the spindle checkpoint, leading to the formation of dyads. Rec8 is required for dyad formation because in its absence MI segregation becomes equational (Watanabe and Nurse 1999). In this equational division sister chromatids segregate from each other with high fidelity, suggesting that in the absence of Rec8 SCC is maintained until MI, perhaps by Rad21. Therefore, the absence of Rec8 restores tension to the MI spindle in a *rec12* mutant background, the spindle checkpoint is not activated, and dyads are not formed. This idea is supported by results in *S. cerevisiae* showing that the spindle checkpoint is activated during *spo11*Δ meioses (Shonn *et al.* 2000). Additionally, expression of a nondegradable form of the anaphase inhibitor Pds1 during meiosis in *S. cerevisiae* leads to the formation of dyad asci. The authors suggested that a fixed time exists between formation of the MI spindle and spore formation and that delaying MI results in spore formation before the completion of MII (Shonn *et al.* 2000). This fits well with the observation that in *rec7* mutants the MI division lasts, on average, more than twice as long as it lasts in wild type (Molnar *et al.* 2001a). Finally, the frequent occurrence in *rec12*Δ dyads of spores that enclose two nuclei (Figure 4C) suggests that at least in some *rec12*Δ meioses MII and spore formation may occur concurrently.

Second, in the absence of recombination Rec8 may not be properly removed from chromosomes, thus delaying or preventing the separation of sister chromatids at MII. In this scenario the incomplete penetrance of dyad formation in *rec12* mutants indicates that the Rec8 remaining on the chromosomes is not always able to resist the pulling forces exerted by the spindle. These possibilities are not mutually exclusive, especially as cleavage of Rec8 may be one target of the spindle checkpoint in meiosis.

Chromosome segregation errors at MI in *rec12*Δ mutants—uneven distribution of DNA and lagging chromosomes: The uneven distribution of DNA between the two MI poles that we observed in *rec12*Δ *mes1*Δ zygotes (Figure 4, H and I) is consistent with the occurrence of chromosome missegregation at MI. Uneven distribution of DNA at MI has also been observed in *rec7*, *rec14*, and *rec15* mutants (Molnar *et al.* 2001a). While this analysis does not distinguish between homolog missegregation and precocious separation of sister chromatids, using a *lacO*-tagged ChrI we showed that in *rec12*Δ meioses, as in *rec*$^+$, sister chromatids segregate to the same pole at MI (Table 6). The uneven distribution of DNA is therefore likely to reflect homolog missegregation.

Lagging chromosomes were occasionally observed in *rec12*Δ *mes1*Δ zygotes (Figure 4I). Lagging chromosomes have also been observed in *rec7*, *rec14*, and *rec15* mutants where chromosomes were observed to move back and forth between the poles during MI (Molnar *et al.* 2001a). Importantly, lagging chromosomes have been observed in many *S. pombe* mutants that perturb mitotic chromosome segregation (Pidoux *et al.* 2000 and references therein; Garcia *et al.* 2002; Rajagopalan and Balasubramanian 2002). At least in some of these mutants the lagging chromosomes also move back and forth between the poles (Pidoux *et al.* 2000). This is thought to be the result of either an unstable connection between chromosomes and spindles or the attachment of chromosomes to spindles from both poles. While this could also be the cause of the lagging chromosomes seen in the absence of recombination, it is possible, as proposed by Molnar *et al.* (2001a), that these chromosome movements represent an active part of a mechanism for achiasmate segregation.

The expected frequency of aneuploid meiotic products as a framework for the interpretation of both genetic and *lacO* segregation data: We have calculated the frequency of viable spores, heterozygous diploids, and ChrIII disomes expected from reductional segregation rates of 50% (random) to 100% (Figure 3). The calculations used to derive Figure 3 were based on four assumptions:

1. The only type of segregation error is MI homolog missegregation. Sister-chromatid cohesion appears normal in *rec12*Δ mutants (Nabeshima *et al.* 2001). Our data support this conclusion; the frequency of precocious separation of ChrI sister chromatids in *rec12*Δ mutants was not significantly different from that in *rec*$^+$ (Table 6). As we mentioned above, two-spored (dyad) asci were common (Figure 4, B and C; Table 3). These frequently were the products of a single reductional division (Table 4). The frequent occurrence in these dyads of spores containing two

nuclei (Figure 4C) suggests that the coordination of MII and spore formation may be perturbed in *rec12*Δ meiosis. Although only half as many spores are produced in a dyad, the types and proportions of aneuploid products produced by MI missegregation will be preserved, in this case with twice as much DNA. As long as dyad formation is random and not biased by the presence or absence of any MI missegregation events, and to the extent that a $2n + 2$ aneuploid behaves like a $1n + 1$ and a $4n$ tetraploid behaves like a $2n$ diploid, our calculations remain valid. Additionally, lagging chromosomes were occasionally observed in *rec12*Δ *mes1*Δ meioses (Figure 4I). This could result in meiotic chromosome loss and decrease spore viability accordingly. However, as long as chromosome loss is random and not biased by the presence or absence of any other MI missegregation event, the calculated frequencies of heterozygous diploids and ChrIII disomes among viable spores remain valid.

2. Missegregation of homologs does not preclude microscopically visible spore formation. We infer from the Hoechst 33342 staining of asci (Figure 4) that visible spores are formed that contain only one or two chromosomes. If MI homolog segregation were completely random, only 1.6% ($= 2^{-6}$, the reciprocal of the number of ways that three pairs of objects can be arranged in two groups) of MII spindle poles would be expected to be nullosomic for all three chromosomes. Therefore, even if completely nullosomic MII spindle poles do not form spores, it is likely an insignificant source of error. Additionally, although a few zygotes did not form visible spores (data not shown), we think that encapsulation of meiotic products into spores is essentially unbiased by the presence or absence of MI missegregation events. Absence of bias is suggested by the internal consistency of the *lacO* segregation data (Table 7), which are not dependent on encapsulation into spores, the calculations of aneuploid frequencies (Figure 3), and the corresponding genetic data (Table 5).
3. All three homologs missegregate with equal frequency. As our chromosome-specific cytological examination of segregation included only ChrI, we have no direct support for this assumption. However, the genetic data (Tables 3–5) are consistent with the reductional segregation of all three chromosomes occurring more frequently than random segregation predicts.
4. ChrIII disomes ($1n + 1$) are viable, while ChrI and ChrII disomes are inviable. ChrIII disomes can be propagated but are unstable (Niwa and Yanagida 1985). Consequently, we might underestimate their frequency. Additionally, although ChrI and ChrII disomes cannot be propagated, they occasionally germinate and can infrequently lose the extra chromosome and resolve to euploidy (Niwa and Yanagida 1985; Molnar *et al.* 2001b). The occasional germination and loss of the extra chromosome in ChrII and ChrIII disomes would result in a euploid spore colony from a meiotic product that we assumed would be inviable.

These possibilities seem not to contribute significantly to the frequency of heterozygous ChrIII disomes or to spore viability for the following reasons: *rec8* mutant meioses, which consist of an equational MI followed by a random MII (Watanabe and Nurse 1999), should yield the same frequency and classes of aneuploids as we calculated for a random MI followed by an equational MII. Our data for *rec8*Δ and *rec8*Δ *rec12*Δ (Table 5) are consistent with this notion. These data also suggest that we were able to efficiently detect unstable heterozygous ChrIII disomes: random MI segregation predicts 30.7% ChrIII disomes (Figure 3), whereas 21.7 ± 2.0% were observed in *rec8*Δ meioses and 30.0 ± 1.3% in *rec8*Δ *rec12*Δ meioses (Table 5). The data also suggest that any viability of ChrI and ChrII disomes does not contribute significantly to spore viability: random MI segregation predicts 20.3% viability (Figure 3), whereas spore viability in *rec8*Δ and *rec8*Δ *rec12*Δ meioses, normalized to wild type, was ~21 and 24%, respectively (Table 5). These considerations strongly suggest that, despite deviations from these assumptions, our calculations remain valid. Therefore, the differences between the observed values for *rec12* mutants and the values predicted for random segregation indicate MI homolog segregation is nonrandom in *rec12* mutants.

Genetic analysis of meiotic segregation in the absence of recombination: To determine whether, in the absence of recombination, homologs segregate randomly at MI, the observed values for *rec12*Δ and *rec12*(Y98F) mutant meioses from Table 5 were plotted onto the corresponding expectation curves (Figure 3). The observed values were most consistent with nonrandom segregation of homologs at MI in the absence of recombination. While random segregation predicts that 7.7% of viable spores will be heterozygous diploids, only 1.3 ± 0.2% and 1.4 ± 0.2% of the viable spores produced in *rec12*Δ and *rec12*(Y98F) meioses, respectively, were heterozygous diploids (Spo^+, Table 5). Additionally, while random segregation predicts that 30.7% of viable spores will be heterozygous ChrIII disomes, 10.5 ± 1.2% of *rec12*Δ and 19.3 ± 2.0% of *rec12*(Y98F) viable meiotic products were heterozygous ChrIII disomes (Table 5). Finally, random segregation predicts that 20.3% of spores will be viable. Spore viability in *rec12*Δ and *rec12*(Y98F) meioses, normalized to wild type, was ~21 and 31%, respectively (Table 5). All of these values, except *rec12*Δ spore viability, are consistent with nonrandom segregation at MI in the absence of recombination.

Our data also suggest that Rec12 and Rec8 independently promote homolog segregation. Rec8, a meiosis-

specific homolog of the sister-chromatid cohesin Scc1 (Parisi *et al.* 1999), promotes sister-chromatid cohesion (Molnar *et al.* 1995). In the absence of Rec8, the first meiotic division is equational rather than reductional (Watanabe and Nurse 1999). This, together with protein localization data, led to the proposal that Rec8 orients kinetechores in such a way that sister chromatids move to the same pole at MI (*i.e.*, homologs segregate; Watanabe and Nurse 1999). *rec12*Δ *rec8*Δ meioses produced significantly more heterozygous ChrIII disomic spores than *rec12*Δ or *rec8*Δ meioses ($P < 0.01$; Table 5), indicating that Rec12 and Rec8 independently promote homolog segregation. These data suggest that recombination may promote a small but significant amount of sister-chromatid cohesion in the absence of Rec8, at least on ChrIII. A similar role for recombination has been proposed in *S. cerevisiae* (Rutkowski and Esposito 2000).

Visualization of a fluorescently tagged ChrI indicates that homologs segregate nonrandomly at MI in *rec12*Δ mutants: To confirm that sister chromatids did not separate precociously in the absence of recombination and that homolog segregation was nonrandom, a *lacO*/GFP-LacI-NLS-marked ChrI was used. Additionally, this system extends our observations to ChrI and allows all meioses to be analyzed, not just those that result in viable spores. We could therefore determine whether the genetic results above were the result of the preferential encapsulation into spores of the products of properly executed meiotic divisions.

First, using crosses heterozygous for the *lacO* array, we showed that sister chromatids did not separate prior to MI in *rec12*Δ *mes1*Δ mutants (Table 6). This is in agreement with previous results in *rec12*Δ *mes*$^+$ meioses (Nabeshima *et al.* 2001). Most importantly, using crosses homozygous for the *lacO* array, we clearly demonstrated that in *rec12*Δ mutants ChrI homologs segregate reductionally at MI 63% of the time (Table 7). This is significantly different from 50% ($\chi^2 = 21$: $P < 0.001$), the frequency predicted by random segregation of homologs, and in close agreement with recent results in the strongly recombination-deficient *S. pombe rec7* mutant (Molnar *et al.* 2001a). Additionally, the value of 63% proper MI segregation is bracketed by the genetic segregation data plotted onto the corresponding expectation curves (Figure 3). This strongly suggests that the genetic results reflect MI segregation rather than a bias in spore production.

Rec12-independent recombination cannot account for the observed level of proper homolog segregation: Extrapolating from our recombination data (Table 8), the genetic length of ChrI in *rec12*Δ mutants is 2.5 cM. Assuming that all of the recombination events were crossovers, this is equivalent to 5% of meioses having one or more crossovers on ChrI. This is not enough to explain the observed degree of nonrandom segregation: to account for 63% reductional division, one or more crossover events would be needed in 26% of meioses to direct segregation. We cannot exclude the possibility that recombination in the telomere proximal regions that we did not measure directs segregation in >20% of *rec12*Δ mutant meioses. However, the 0.5 Mb of ChrI that was not analyzed would need to have a recombination intensity (genetic distance/physical distance) 40 times greater than that of the 5.1 Mb we analyzed. This seems unlikely. Instead, we favor the idea that a mechanism exists in *S. pombe* that promotes proper reductional segregation of homologs at MI independently of recombination.

This conclusion is seemingly at odds with the conclusion of Niwa *et al.* (1989), who observed that two minichromosomes that did not recombine segregated from each other at MI only 56% of the time. These data suggest that *S. pombe* does not possess a segregation system similar to that of *S. cerevisiae*, which can segregate achiasmate minichromosomes properly ~90% of the time (Dawson *et al.* 1986). However, because only 39 informative meioses were examined, the data of Niwa *et al.* (1989) are not significantly different from our *lacO* data (Table 7; $\chi^2 = 0.75$: $0.25 < P < 0.5$).

Nonrandom segregation at MI does not require a function of Rec12 distinct from the promotion of DNA double-strand breaks: Our limited characterization of the strong *rec* mutants *rec6*, *rec7*, *rec14*, and *rec15* showed an increased frequency of diploid spores and a low level of heterozygous diploids (Spo$^+$) similar to *rec12* mutants (Table 9). These data are consistent with both genetic and cytological experiments in *rec7* mutants by the Kohli and Hiraoka labs (Molnar *et al.* 2001a,b) and suggest that the segregation phenotypes in *rec12* mutant meioses are the result of the absence of recombination rather than of some other function of Rec12. Taken at face value, the difference between the frequency of ChrIII disomes produced by *rec12*Δ and *rec12*(Y98F) meioses (10.5 ± 1.2% and 19.3 ± 2.0%, respectively) contradicts this interpretation. Contrary to the suggested recombination-independent role in stabilizing homologous chromosome interactions of other Spo11 (Rec12) homologs in other organisms (Cha *et al.* 2000; Romanienko and Camerini-Otero 2000), our results would suggest that *rec12*(Y98F) inhibits, rather than promotes, proper MI segregation. However, our analysis of the Ade$^+$ products of *rec12*(Y98F)/*rec12*Δ heterozygous meiosis argues against this simple interpretation. The frequency of Ade$^+$ products in the heterozygous meioses is intermediate to the levels from the *rec12*Δ and *rec12*(Y98F) homozygous meioses (Table 5). Interestingly, more than half of the Ade$^+$ products from the heterozygous meioses were *rec12*(Y98F) segregants, and the Ade$^+$ *rec12*Δ (*rec12-169::3HA6His-kanMX6*) segregants predominantly formed smaller colonies than the Ade$^+$ *rec12*(Y98F) segregants (data not shown). We note, however, that there is no obvious growth defect in the *rec12*Δ haploid parents (data not shown). These results suggest that the

germination or mitotic growth of *rec12*Δ Ade$^+$ heterozygous ChrIII disomes is perturbed. Unfortunately, this suggested perturbation prevents us from comparing our results with recent, seemingly contradictory, results (Sharif *et al.* 2002). After analyzing a different subset of meiotic products from what we used, these authors suggested that, while both *rec12* null and Y98F mutants segregate homologs nonrandomly at MI, Y98F mutants segregate properly more frequently than the null mutants.

The discrepancy we observed in the frequency of ChrIII disomes produced by *rec12*Δ and *rec12*(Y98F) meioses is not fully understood. However, all of the genetic data for *rec12*(Y98F) tightly bracket the frequency of homolog missegregation as assayed by *lacO* in *rec12*Δ mutants (Figure 3). This is consistent with the hypothesis that segregation is equivalent in *rec12*Δ and *rec12*(Y98F), and only the ability to form ChrIII disomic colonies is perturbed in *rec12*Δ. Whatever the reason for the difference between *rec12*Δ and *rec12*(Y98F), it does not alter the main conclusion of this work. The frequency of heterozygous ChrIII disomic spore colonies is significantly less than that expected from random segregation for both *rec12*Δ and *rec12*(Y98F). Therefore, both results are consistent with the hypothesis that in the absence of meiotic recombination, all three chromosomes segregate reductionally more frequently than random segregation predicts.

The properties of the *S. pombe* achiasmate segregation system differ from those of achiasmate segregation systems described in other organisms: The achiasmate system in *S. cerevisiae* achieves ~90% proper MI segregation of two achiasmate artificial chromosomes (Dawson *et al.* 1986) or of two monosomic nonhomologous chromosomes (Guacci and Kaback 1991). However, *S. cerevisiae* is unable to promote nonrandom segregation when meiotic recombination is abolished throughout the genome. Segregation of ChrV at MI is random in *spo11* mutant meioses (Table 1 in Klein *et al.* 1999). Although a small deviation from random (50%) would not have been detected, the *spo11* data are significantly different from the 63% that we observed using the *lacO*-marked ChrI ($\chi^2 = 11$: $P < 0.005$). Our results demonstrate that when meiotic recombination is abolished throughout the genome by mutation of *rec12*, MI reductional segregation of ChrI is nonrandom. Perhaps because *S. pombe* chromosomes are much longer than those in *S. cerevisiae* or because *S. pombe* chromosomes possess more centric heterochromatin (Kniola *et al.* 2001; reviewed in Pidoux and Allshire 2000), early homolog interactions may be more readily stabilized by the achiasmate segregation system in *S. pombe*. Alternatively, the only difference may be that the achiasmate segregation system in *S. cerevisiae* is overwhelmed by the large number of achiasmate chromosomes (16 *vs.* 3 in *S. pombe*).

Unlike the achiasmate segregation system in *S. pombe*, the segregation systems in both male and female Drosophila are called upon to segregate achiasmate chromosomes in every meiosis. These systems are able to ensure nearly 100% proper MI segregation of naturally occurring achiasmate chromosomes. In males, where all chromosomes are achiasmate, stable pairing requires a specific region of heterochromatin on the sex chromosomes and euchromatin on the autosomes (reviewed in McKee 1998). Perhaps the initial homolog interactions are stabilized and effectively able to oppose the poleward forces exerted by the spindle apparatus, thus producing tension and stabilizing bipolar attachment of homologs.

Drosophila females possess two distinct achiasmate segregation systems (reviewed in Hawley and Theurkauf 1993). In the first, pairing and segregation of achiasmate homologs require homology between centromere-proximal heterochromatin (Dernburg *et al.* 1996; Karpen *et al.* 1996). Pairing of the centric heterochromatin, but not of the euchromatin, is maintained until the spindle is assembled (Dernburg *et al.* 1996). In the second, two achiasmate heterologous chromosomes also segregate from each other with high fidelity. In this case pairing is not observed (Dernburg *et al.* 1996), and the heterologous chromosomes are thought to orient to the less crowded pole, thus assuring that one will go to each pole. It is interesting to note that Meu13, a homolog of the *S. cerevisiae* Hop2 protein, contributes to a recombination-independent pairing process in *S. pombe* (Nabeshima *et al.* 2001): Meu13-dependent pairing was observed in *rec12* mutant meiosis, although at a level lower than that in wild type. Perhaps this recombination-independent, Meu13-dependent pairing is important for nonrandom segregation of homologs at MI in *rec12* mutant meiosis. It seems likely that this recombination-independent pairing requires homology. However, it is possible that the initial contacts between homologs promoted by telomere clustering and nuclear movement (reviewed in Yamamoto and Hiraoka 2001) are stabilized independently of homology.

While the mechanism that brings about nonrandom MI segregation of *S. pombe* chromosomes in the absence of recombination is still uncharacterized, we suggest that it differs from those in Drosophila in at least one important way. *S. pombe* chromosomes, like those in both *S. cerevisiae* and humans, do not generally fail to recombine in a significant fraction of meioses. In *S. pombe* ChrIII, the shortest chromosome, receives ~11 crossovers on average and, if this number is Poisson distributed, is expected to fail to undergo a crossover event in 2×10^{-5} meioses (Munz 1994). The shortest *S. cerevisiae* chromosome, ChrI, has a genetic length of 127 cM and has been estimated to fail to undergo a crossover event in 2–8 $\times 10^{-3}$ meioses (Kaback *et al.* 1989). Estimates for the frequency of human chromosome 21 failing to undergo a crossover event range from 1×10^{-3} to 7×10^{-2} (reviewed in Koehler and Hassold 1998). The

achiasmate segregation systems in these yeasts, and perhaps in humans if one is present, are not called upon to promote the segregation of achiasmate chromosomes in every meiosis. Perhaps for this reason the *S. pombe* and *S. cerevisiae* systems do not ensure the high degree of proper MI segregation of achiasmate chromosomes that is seen in Drosophila. Rather than a dedicated system designed specifically to ensure the segregation of achiasmate chromosomes, it seems likely that nonrandom segregation of achiasmate chromosomes may be a result of the specific nature of early homolog interactions in these organisms. The study of achiasmate chromosome segregation in *S. pombe* may lead to a better understanding of the types of interactions that initiate the pairing of homologs as well as factors that stabilize these interactions.

We are grateful to Jürg Kohli, Andrew Murray, Kentaro Nabeshima, Mitsuhiro Yanagida, Paul Russell, and Chikashi Shimoda for providing the *rec8::ura4*$^+$ allele, GFP-LacI-NLS plasmids, *lacO* array, *spc1::ura4*$^+$ allele, and *mes1::LEU2* allele, respectively; and to Sue Amundson, Sue Biggins, Joseph Farah, Walter Steiner, Andrew Taylor, and two anonymous reviewers for helpful comments on the manuscript. This work was supported by National Institutes of Health grants R01-GM32194 to G.R.S. and F32-GM20125 to L.D.

LITERATURE CITED

Apolinario, E., M. Nocero, M. Jin and C. S. Hoffman, 1993 Cloning and manipulation of the *Schizosaccharomyces pombe his7*$^+$ gene as a new selectable marker for molecular genetic studies. Curr. Genet. **24:** 491–495.

Bahler, J., J. Q. Wu, M. S. Longtine, N. G. Shah, A. McKenzie, III *et al.*, 1998 Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast **14:** 943–951.

Bergerat, A., B. de Massy, D. Gadelle, P. C. Varoutas, A. Nicolas *et al.*, 1997 An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature **386:** 414–417.

Bernard, P., J. F. Maure and J. P. Javerzat, 2001 Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. Nat. Cell Biol. **3:** 522–526.

Bresch, C., G. Muller and R. Egel, 1968 Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. **102:** 301–306.

Buonomo, S. B., R. K. Clyne, J. Fuchs, J. Loidl, F. Uhlmann *et al.*, 2000 Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell **103:** 387–398.

Cervantes, M. D., J. A. Farah and G. R. Smith, 2000 Meiotic DNA breaks associated with recombination in *S. pombe*. Mol. Cell **5:** 883–888.

Cha, R. S., B. M. Weiner, S. Keeney, J. Dekker and N. Kleckner, 2000 Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev. **14:** 493–503.

Cooper, J. P., Y. Watanabe and P. Nurse, 1998 Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. Nature **392:** 828–831.

Cummins, J. E., and J. M. Mitchison, 1967 Adenine uptake and pool formation in the fission yeast *Schizosaccharomyces pombe*. Biochim. Biophys. Acta **136:** 108–120.

Dawson, D. S., A. W. Murray and J. W. Szostak, 1986 An alternative pathway for meiotic chromosome segregation in yeast. Science **234:** 713–717.

Dernburg, A. F., J. W. Sedat and R. S. Hawley, 1996 Direct evidence of a role for heterochromatin in meiotic chromosome segregation. Cell **86:** 135–146.

DeVeaux, L. C., and G. R. Smith, 1994 Region-specific activators of meiotic recombination in *Schizosaccharomyces pombe*. Genes Dev. **8:** 203–210.

DeVeaux, L. C., N. A. Hoagland and G. R. Smith, 1992 Seventeen complementation groups of mutations decreasing meiotic recombination in *Schizosaccharomyces pombe*. Genetics **130:** 251–262.

Evans, D. H., Y. F. Li, M. E. Fox and G. R. Smith, 1997 A WD repeat protein, Rec14, essential for meiotic recombination in *Schizosaccharomyces pombe*. Genetics **146:** 1253–1264.

Forsburg, S. L., 1993 Comparison of *Schizosaccharomyces pombe* expression systems. Nucleic Acids Res. **21:** 2955–2956.

Garcia, M. A., N. Koonrugsa and T. Toda, 2002 Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A. Curr. Biol. **12:** 610–621.

Grallert, B., and M. Sipiczki, 1989 Initiation of the second meiotic division in *Schizosaccharomyces pombe* shares common functions with that of mitosis. Curr. Genet. **15:** 231–233.

Grallert, B., and M. Sipiczki, 1991 Common genes and pathways in the regulation of the mitotic and meiotic cell cycles of *Schizosaccharomyces pombe*. Curr. Genet. **20:** 199–204.

Grimm, C., J. Kohli, J. Murray and K. Maundrell, 1988 Genetic engineering of *Schizosaccharomyces pombe*: a system for gene disruption and replacement using the *ura4* gene as a selectable marker. Mol. Gen. Genet. **215:** 81–86.

Guacci, V., and D. B. Kaback, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. Genetics **127:** 475–488.

Gutz, H., H. Heslot, U. Leupold and N. Loprieno, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics*, edited by R. C. King. Plenum Press, New York.

Hassold, T., and P. Hunt, 2001 To err (meiotically) is human: the genesis of human aneuploidy. Nat. Rev. Genet. **2:** 280–291.

Hawley, R. S., 1989 Exchange and chromosomal segregation in eucaryotes, pp. 497–525 in *Genetic Recombination*, edited by R. Kucherlapati and G. R. Smith. American Society for Microbiology, Washington, DC.

Hawley, R. S., and W. E. Theurkauf, 1993 Requiem for distributive segregation: achiasmate segregation in *Drosophila* females. Trends Genet. **9:** 310–317.

Kaback, D. B., H. Y. Steensma and P. de Jonge, 1989 Enhanced meiotic recombination on the smallest chromosome of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **86:** 3694–3698.

Karpen, G. H., M. H. Le and H. Le, 1996 Centric heterochromatin and the efficiency of achiasmate disjunction in Drosophila female meiosis. Science **273:** 118–122.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kishida, M., T. Nagai, Y. Nakaseko and C. Shimoda, 1994 Meiosis-dependent mRNA splicing of the fission yeast *Schizosaccharomyces pombe mes1*$^+$ gene. Curr. Genet. **25:** 497–503.

Klein, F., P. Mahr, M. Galova, S. B. Buonomo, C. Michaelis *et al.*, 1999 A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell **98:** 91–103.

Kniola, B., E. O'Toole, J. R. McIntosh, B. Mellone, R. Allshire *et al.*, 2001 The domain structure of centromeres is conserved from fission yeast to humans. Mol. Biol. Cell **12:** 2767–2775.

Koehler, K. E., and T. J. Hassold, 1998 Human aneuploidy: lessons from achiasmate segregation in *Drosophila melanogaster*. Ann. Hum. Genet. **62:** 467–479.

Kohli, J., H. Hottinger, P. Munz, A. Strauss and P. Thuriaux, 1977 Genetic mapping in *Schizosaccharomyces pombe* by mitotic and meiotic analysis and induced haploidization. Genetics **87:** 471–489.

Lichten, M., 2001 Meiotic recombination: breaking the genome to save it. Curr. Biol. **11:** R253–R256.

Lin, Y., and G. R. Smith, 1994 Transient, meiosis-induced expression of the *rec6* and *rec12* genes of *Schizosaccharomyces pombe*. Genetics **136:** 769–779.

Lin, Y., and G. R. Smith, 1995 An intron-containing meiosis-induced recombination gene, *rec15*, of *Schizosaccharomyces pombe*. Mol. Microbiol. **17:** 439–448.

Lin, Y., K. L. Larson, R. Dorer and G. R. Smith, 1992 Meiotically induced *rec7* and *rec8* genes of *Schizosaccharomyces pombe*. Genetics **132:** 75–85.

McKee, B. D., 1998 Pairing sites and the role of chromosome pairing

in meiosis and spermatogenesis in male Drosophila. Curr. Top. Dev. Biol. **37:** 77–115.

Merino, S. T., W. J. Cummings, S. N. Acharya and M. E. Zolan, 2000 Replication-dependent early meiotic requirement for Spo11 and Rad50. Proc. Natl. Acad. Sci. USA **97:** 10477–10482.

Molnar, M., and M. Sipiczki, 1993 Polyploidy in the haplontic yeast *Schizosaccharomyces pombe*: construction and analysis of strains. Curr. Genet. **24:** 45–52.

Molnar, M., J. Bahler, M. Sipiczki and J. Kohli, 1995 The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. Genetics **141:** 61–73.

Molnar, M., J. Bahler, J. Kohli and Y. Hiraoka, 2001a Live observation of fission yeast meiosis in recombination-deficient mutants: a study on achiasmate chromosome segregation. J. Cell Sci. **114:** 2843–2853.

Molnar, M., S. Parisi, Y. Kakihara, H. Nojima, A. Yamamoto *et al.*, 2001b Characterization of *rec7*, an early meiotic recombination gene in *Schizosaccharomyces pombe*. Genetics **157:** 519–532.

Munz, P., 1994 An analysis of interference in the fission yeast *Schizosaccharomyces pombe*. Genetics **137:** 701–707.

Munz, P., K. Wolf, J. Kohli and U. Leupold, 1989 Genetics overview, pp. 1–30 in *Molecular Biology of the Fission Yeast*, edited by A. Nasim, P. Young and B. F. Johnson. Academic Press, San Diego.

Nabeshima, K., H. Kurooka, M. Takeuchi, K. Kinoshita, Y. Nakaseko *et al.*, 1995 p93dis1, which is required for sister chromatid separation, is a novel microtubule and spindle pole body-associating protein phosphorylated at the Cdc2 target sites. Genes Dev. **9:** 1572–1585.

Nabeshima, K., T. Nakagawa, A. F. Straight, A. Murray, Y. Chikashige *et al.*, 1998 Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle. Mol. Biol. Cell **9:** 3211–3225.

Nabeshima, K., Y. Kakihara, Y. Hiraoka and H. Nojima, 2001 A novel meiosis-specific protein of fission yeast, Meu13p, promotes homologous pairing independently of homologous recombination. EMBO J. **20:** 3871–3881.

Nakaseko, Y., O. Niwa and M. Yanagida, 1984 A meiotic mutant of the fission yeast *Schizosaccharomyces pombe* that produces mature asci containing two diploid spores. J. Bacteriol. **157:** 334–336.

Nasmyth, K., 2001 Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. Annu. Rev. Genet. **35:** 673–745.

Nicklas, R. B., 1997 How cells get the right chromosomes. Science **275:** 632–637.

Nimmo, E. R., A. L. Pidoux, P. E. Perry and R. C. Allshire, 1998 Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. Nature **392:** 825–828.

Niwa, O., and M. Yanagida, 1985 Triploid meiosis and aneuploidy in *Schizosaccharomyces pombe*: an unstable aneuploid disomic for chromosome III. Curr. Genet. **9:** 463–470.

Niwa, O., and M. Yanagida, 1988 Universal and essential role of MPF/*cdc2*$^+$. Nature **336:** 430.

Niwa, O., T. Matsumoto, Y. Chikashige and M. Yanagida, 1989 Characterization of Schizosaccharomyces pombe minichromosome deletion derivatives and a functional allocation of their centromere. EMBO J. **8:** 3045–3052.

Nurse, P., 1975 Genetic control of cell size at cell division in yeast. Nature **256:** 547–551.

Parisi, S., M. J. McKay, M. Molnar, M. A. Thompson, P. J. van der Spek *et al.*, 1999 Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. Mol. Cell. Biol. **19:** 3515–3528.

Pidoux, A. L., and R. C. Allshire, 2000 Centromeres: getting a grip of chromosomes. Curr. Opin. Cell Biol. **12:** 308–319.

Pidoux, A. L., S. Uzawa, P. E. Perry, W. Z. Cande and R. C. Allshire, 2000 Live analysis of lagging chromosomes during anaphase and their effect on spindle elongation rate in fission yeast. J. Cell Sci. **113:** 4177–4191.

Ponticelli, A. S., and G. R. Smith, 1989 Meiotic recombination-deficient mutants of *Schizosaccharomyces pombe*. Genetics **123:** 45–54.

Rajagopalan, S., and M. K. Balasubramanian, 2002 *Schizosaccharomyces pombe* Bir1p, a nuclear protein that localizes to kinetochores and the spindle midzone, is essential for chromosome condensation and spindle elongation during mitosis. Genetics **160:** 445–456.

Romanienko, P. J., and R. D. Camerini-Otero, 2000 The mouse Spo11 gene is required for meiotic chromosome synapsis. Mol. Cell **6:** 975–987.

Rutkowski, L. H., and R. E. Esposito, 2000 Recombination can partially substitute for *SPO13* in regulating meiosis I in budding yeast. Genetics **155:** 1607–1621.

Scherthan, H., 2001 A bouquet makes ends meet. Nat. Rev. Mol. Cell Biol. **2:** 621–627.

Sharif, W., G. Glick, M. Davidson and W. Wahls, 2002 Distinct functions of *S. pombe* Rec12 (Spo11) protein and Rec12-dependent crossover recombination (chiasmata) in meiosis I; and a requirement for Rec12 in meiosis II. Cell Chromosome **1:** 1–14.

Shimanuki, M., F. Miki, D. Q. Ding, Y. Chikashige, Y. Hiraoka *et al.*, 1997 A novel fission yeast gene, *kms1*$^+$, is required for the formation of meiotic prophase-specific nuclear architecture. Mol. Gen. Genet. **254:** 238–249.

Shimoda, C., A. Hirata, M. Kishida, T. Hashida and K. Tanaka, 1985 Characterization of meiosis-deficient mutants by electron microscopy and mapping of four essential genes in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **200:** 252–257.

Shiozaki, K., and P. Russell, 1995 Cell-cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. Nature **378:** 739–743.

Shonn, M. A., R. McCarroll and A. W. Murray, 2000 Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. Science **289:** 300–303.

Straight, A. F., J. W. Sedat and A. W. Murray, 1998 Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. J. Cell Biol. **143:** 687–694.

Sveiczer, A., B. Novak and J. Mitchison, 1996 The size control of fission yeast revisited. J. Cell Sci. **109:** 2947–2957.

Uhlmann, F., F. Lottspeich and K. Nasmyth, 1999 Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature **400:** 37–42.

Watanabe, Y., and P. Nurse, 1999 Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature **400:** 461–464.

Watanabe, Y., S. Yokobayashi, M. Yamamoto and P. Nurse, 2001 Pre-meiotic S phase is linked to reductional chromosome segregation and recombination. Nature **409:** 359–363.

Wood, V., R. Gwilliam, M. A. Rajandream, M. Lyne, R. Lyne *et al.*, 2002 The genome sequence of *Schizosaccharomyces pombe*. Nature **415:** 871–880.

Yamamoto, A., and Y. Hiraoka, 2001 How do meiotic chromosomes meet their homologous partners?: lessons from fission yeast. Bioessays **23:** 526–533.

Yamamoto, A., R. R. West, J. R. McIntosh and Y. Hiraoka, 1999 A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. J. Cell Biol. **145:** 1233–1249.

Communicating editor: M. Lichten