Meiotic Chromosome Dynamics Dependent Upon the *rec8*$^+$, *rec10*$^+$ and *rec11*$^+$ Genes of the Fission Yeast *Schizosaccharomyces pombe*

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Manuscript received January 14, 1999
Accepted for publication May 27, 1999

ABSTRACT

During meiosis homologous chromosomes replicate once, pair, experience recombination, and undergo two rounds of segregation to produce haploid meiotic products. The *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes of the fission yeast *Schizosaccharomyces pombe* exhibit similar specificities for meiotic recombination and *rec8*$^+$ is required for sister chromatid cohesion and homolog pairing. We applied cytological and genetic approaches to identify potential genetic interactions and to gauge the fidelity of meiotic chromosome segregation in the mutants. The *rec8*$^+$ gene was epistatic to *rec10*$^+$ and to *rec11*$^+$, but there was no clear epistatic relationship between *rec10*$^+$ and *rec11*$^+$. Reciprocal (crossover) recombination in the central regions of all three chromosomes was compromised in the *rec* mutants, but recombination near the telomeres was nearly normal. Each of the mutants also exhibited a high rate of aberrant segregation for all three chromosomes. The *rec8* mutations affected mainly meiosis I segregation. Remarkably, the *rec10* and *rec11* mutations, which compromised recombination during meiosis I, affected mainly meiosis II segregation. We propose that these genes encode regulators or components of a "meiotic chromatid cohesion" pathway involved in establishing, maintaining, and appropriately releasing meiotic interactions between chromosomes. A model of synergistic interactions between sister chromatid cohesion and crossover position suggests how crossovers and cohesion help ensure the proper segregation of chromosomes in each of the meiotic divisions.

MEIOSIS is a key component of sexual reproduction in eukaryotes. After premeiotic DNA replication, each chromosome is composed of two sister chromatids that remain associated with each other. Homologous chromosomes (two sets of sister chromatids) then pair to form a bivalent and undergo recombination. This is followed by the meiosis I (MI) reductional division, in which homologous chromosomes segregate from each other, and then the meiosis II (MII) equational division, in which sister chromatids segregate from each other to complete meiosis.

Recombination occurs at a high frequency during meiosis and serves to generate genetic diversity and, in most organisms, to help ensure the appropriate segregation of chromosomes at the first meiotic division (Moens 1994; Roeder 1997; Moore and Orr-Weaver 1998). Crossovers (reciprocal recombination events) generate chiasmata, which are cytologically visible nucleoprotein structures that help to stabilize connections between homologous chromosomes so they can orient properly on the MI spindle and thus segregate accurately.

The *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes of fission yeast were identified in a screen for mutations that decrease meiotic recombination at the *ade6* locus (Ponticelli and Smith 1989). Their expression is induced in meiosis (Lin *et al.* 1992; Lin and Smith 1995; Li *et al.* 1997); they are reportedly specific for recombination on chromosome *III* (DeVeaux and Smith 1994), but their primary function may be in some other meiotic process. Upon chromosome synapsis, *Schizosaccharomyces pombe* does not form a tripartite synaptonemal complex (SC), but it does form discontinuous patches of filamentous structures, known as "linear elements," similar to axial elements of SC (Bähler *et al.* 1993; Scherthan *et al.* 1994). The *rec8* mutants do not properly pair the internal regions of homologous chromosomes, they fail to form proper linear elements, and they suffer precocious separation of sister chromatids and nondisjunction of homologous chromosomes during MI (Molnar *et al.* 1995; Krawchuk and Wahls 1999).

After correcting (Parisi *et al.* 1999) for multiple errors in the published coding sequence (Lin *et al.* 1992), *rec8*$^+$ has homology to a family of mitotically expressed genes defined by the *rad21*$^+$ gene of *S. pombe*. The *rad21*$^+$ gene and other *rad21*$^+$ "cohesin" gene family members are required for the proper mitotic transmission of chromosomes (Birkenbihl and Subramani 1992, 1995; McKay *et al.* 1996; Guacci *et al.* 1997; Michaelis *et al.* 1997; Heo *et al.* 1998; Tatebayashi *et al.* 1998). The *rec10*$^+$ gene (Lin and Smith 1995) encodes a pioneer protein with no significant primary sequence homology to proteins or translations of DNA sequences currently present

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in computer databases (unpublished observations). The predicted Rec11 polypeptide has sequence conservation with several proteins of unknown function present in the databases, including a hypothetical protein encoded in *S. pombe* genomic DNA (GenBank accession no. Z98597; our unpublished observations).

We have characterized the roles of the *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes in meiosis. We report that they exhibit epistatic interactions and are variously required for aspects of chromosome dynamics ranging from the initial pairing of homologous chromosomes to chromosome segregation in the second meiotic division. Models in which a "meiotic chromatid cohesion" pathway might participate in these functions are presented.

MATERIALS AND METHODS

***S. pombe* culture:** The *S. pombe* strain genotypes are listed in Table 1. Culture media and genetic methods were as previously described (Gutz *et al.* 1974; Kon *et al.* 1997). Yeast extract agar (YEA)-B was YEA containing 2.5 μg/ml of Phloxin-B (Sigma, St. Louis) and 100 μg/ml of adenine. Synthetic sporulation agar (SPA), nitrogen base agar (NBA), and nitrogen base liquid (NBL) were supplemented with required amino acids, adenine, and uracil at 100 μg/ml.

Recombinant frequency determination: Mating, meiosis, and preparation of free spores were as previously described (Gutz *et al.* 1974; Kon *et al.* 1997). Intergenic (crossover) recombination was measured by plating spores on media with various combinations of growth factors to select for double prototrophic recombinants. The recombinant frequency was calculated relative to the viable spore titer. Because diploid spores could contain complementing markers and be mistaken for recombinants, we tested all spore colonies for diploidy by replica plating to media containing Phloxin-B (YEA-B). On Phloxin-B-containing plates, haploid cells produce light pink colonies whereas diploid cells produce dark pink colonies (Gutz *et al.* 1974). Diploid meiotic products were excluded from recombinant frequency determinations (below; Table 4).

Diploid spore isolation and haploidization analysis: Spores were plated on YEA-B and incubated for 3 days at 32° to distinguish the diploid spore colonies from the haploid spore colonies. The ploidy was confirmed by microscopic examination of the cells. Diploid cells are both longer and wider

TABLE 1

***S. pombe* strains**

Strain	Genotype
WSP 15[a]	*h*$^+$ *leu1-32*
WSP 20[a]	*h*$^+$ *ade6-M26 pat1-114*
WSP 51[a]	*h*$^-$ *ade6-52 pat1-114 rec8-110*
WSP 52[a]	*h*$^-$ *ade6-52 rec11-111 pat1-114*
WSP 58[a]	*h*$^-$ *ade6-52 pat1-114 end1-458 rec10-109*
WSP 71[a]	*h*$^+$ *ade6-52 pat1-114 end1-458 leu1-32 rec11-111*
WSP 158[a]	*h*$^-$ *pro2-1*
WSP 162[a]	*h*$^-$ *leu2-120*
WSP 164[a]	*h*$^-$ *arg6-328*
WSP 165[a]	*h*$^+$ *arg6-328*
WSP 208[a]	*h*$^-$ *ade6-M26 ura4-294 arg3-124*
WSP 213[a]	*h*$^+$ *ade6-52 ura4-595 pro2-1*
WSP 306[b]	*h*$^-$ *ade6-52 ura4-595 pro2-1 rec10-109*
WSP 307[c]	*h*$^+$ *ade6-52 ura4-595 pro2-1 rec8-110*
WSP 308[b]	*h*$^-$ *ade6-52 ura4-595 pro2-1 rec11-111*
WSP 310[b]	*h*$^+$ *ade6-M26 ura4-294 arg3-124 rec10-109*
WSP 311[d]	*h*$^-$ *ade6-M26 ura4-294 arg3-124 rec8-110*
WSP 312[b]	*h*$^+$ *ade6-M26 ura4-294 arg3-124 rec11-111*
WSP 321[e]	*h*$^-$ *ade6-52 ura4-294 pro2-1 rec10-109 rec11-111*
WSP 325[e]	*h*$^+$ *ade6-M26 ura4-294 arg3-124 rec10-109 rec11-111*
WSP 356	*h*$^-$ *ade6-52 his5-303*
WSP 365[d]	*h*$^-$ *ade6-52 ura4-595 pro2-1 rec8-110*
WSP 367[c]	*h*$^+$ *ade6-52 leu1-32 rec8-110*
WSP 389	*h*$^-$ *his1-102*
WSP 418	*h*$^+$ *ade6-M26 ura4-294 arg3-124 rec8-110 rec10-109*
WSP 419	*h*$^-$ *ade6-52 ura4-595 pro2-1 rec8-110 rec10-109*
WSP 420	*h*$^+$ *ade6-M26 ura4-294 arg3-124 rec8-110 rec11-111*
WSP 421	*h*$^-$ *ade6-52 ura4-595 pro2-1 rec8-110 rec11-111*
WSP 517[f]	*h*$^+$ *lys1-131*
WSP 523[f]	*h*$^-$ *his2-1*
WSP 788[g]	*h*$^-$ *his3-D1*
WSP 1149	*h*$^+$ *ade6-M26 lys1-131 rec8-110*
WSP 1150	*h*$^+$ *ade6-M26 lys-1-131 rec10-109*
WSP 1151	*h*$^+$ *ade6-M26 lys1-131 rec11-111*
WSP 1215	*h*$^-$ *ade6-52 tsp13-24 rec8-110*
WSP 1219	*h*$^-$ *ade6-52 tsp13-24 rec10-109*
WSP 1220	*h*$^-$ *ade6-52 tsp13-24 rec11-111*
WSP 1270	*h*$^-$ *ade6-52 leu2-120 tps13-24 rec8-110*
WSP 1271	*h*$^-$ *ade6-52 leu2-120 tps13-24 rec10-109*
WSP 1272	*h*$^-$ *ade6-52 leu2-120 tps13-24 rec11-111*
WSP 1310	*h*$^+$ *ade6-M26 arg6-328 rec8-110*
WSP 1311	*h*$^+$ *ade6-M26 arg6-328 rec11-111*
WSP 1317	*h*$^-$ *ade6-M26 his1-102 rec11-111*
WSP 1319	*h*$^-$ *ade6-M26 his2-1 rec11-111*
WSP 1320	*h*$^+$ *ade6-M26 leu2-135 rec10-109*
WSP 1321	*h*$^+$ *ade6-M26 leu3-135 rec11-111*
WSP 1322	*h*$^-$ *ade6-M26 his3-D1 rec8-110*
WSP 1323	*h*$^-$ *ade6-M26 his3-D1 rec10-109*
WSP 1324	*h*$^-$ *ade6-M26 his3-D1 rec11-111*
WSP 1328	*h*$^-$ *ade6-M26 his1-102 rec8-110*
WSP 1329	*h*$^-$ *ade6-M26 his2-1 rec10-109*
WSP 1385	*h*$^+$ *ade6-M26 leu1-32 rec10-109*
WSP 1389	*h*$^-$ *ade6-M26 his2-1 rec8-110*
WSP 1391	*h*$^+$ *ade6-M26 leu3-135*
WSP 1392	*h*$^+$ *ade6-M26 arg6-328 rec10-109*
WSP 1393	*h*$^-$ *ade6-M26 his5-303 rec8-110*
WSP 1394	*h*$^-$ *ade6-M26 his5-303 rec10-109*
WSP 1398	*h*$^+$ *ade6-M26 his3-D1 rec8-110*
WSP 1399	*h*$^-$ *ade6-M26 his5-303 rec11-111*
WSP 1403	*h*$^+$ *ade6-M26 his3-D1 rec11-111*
WSP 1448	*h*$^-$ *ade6-M26 his1-102 rec10-109*
WSP 1449	*h*$^+$ *ade6-M26 leu3-135 rec8-110*

(*continued*)

TABLE 1

(Continued)

[a] Gerald Smith.
[b] Ponticelli and Smith (1989).
[c] DeVeaux and Smith (1994).
[d] DeVeaux *et al.* (1992).
[e] Nancy Hollingsworth.
[f] Kathy Gould.
[g] Jürg Kohli.

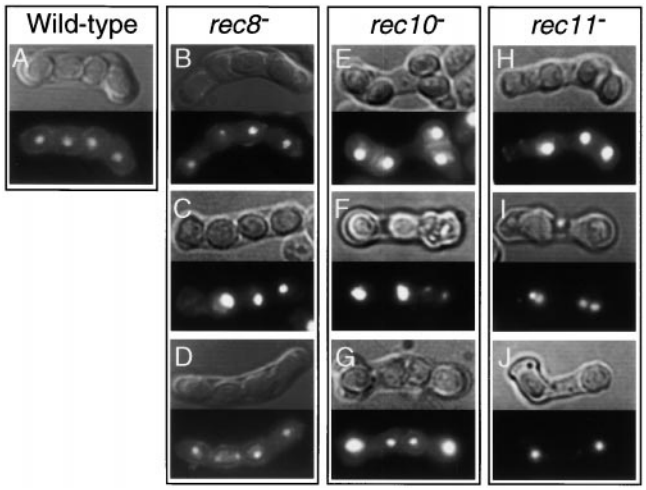


Figure 1.—Representative cytological phenotypes of wild-type and *rec* mutant meiotic products. (A–J) DIC (top) and DAPI fluorescence (bottom) images of asci from (A) wild-type; (B–D) *rec8*; (E–G) *rec10*; and (H–J) *rec11* mutant meioses. The correspondence between each of the cytological phenotypes and their schematic representation is provided in Table 2.

than haploid cells and can be readily distinguished under the microscope.

The parental haploid strains that were crossed had different alleles at loci on the three chromosomes. The genotypes of the resulting diploid spore colonies were determined by analysis of haploidized colonies on diagnostic plates. Diploid spore colonies were haploidized with *m*-fluorophenylalanine (*m*-FPA; Kohli *et al.* 1977). Fifty haploidized colonies derived from each individual diploid spore colony were replica plated to various differentially supplemented minimal media to genotype the *ade6* and *lys1* loci. The *tps13* alleles were scored by replica plating colonies onto rich (YEA) media and scoring for growth at the permissive (22°) and restrictive (35°) temperatures. The *mat1* alleles were determined by test crossing to strains of known mating type and iodine staining of the resultant asci (Gutz *et al.* 1974).

Microscopic analysis: To monitor chromosome segregation, asci from meiotic cultures were fixed with 95% ethanol, washed with PBS, and stained with 4,6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 μg/ml. Cells were spread on poly-l-lysine-coated slides and visualized by differential interference contrast (DIC) and fluorescence microscopy with a Zeiss axiophot (Carl Zeiss, Thornwood, NY). Images were captured using the MetaMorph software package (Universal Imaging, West Chester, PA).

RESULTS

Aberrant meiotic development in the *rec8*, *rec10*, and *rec11* mutants: *S. pombe* is a particularly attractive model organism in which to study meiotic chromosome dynamics because it has only three chromosome pairs and all four products of each meiosis (the spores) are held together in an ascus. To gain insight into the functions of the $rec8^+$, $rec10^+$, and $rec11^+$ genes, asci from meiotic cultures were stained with a DNA-specific fluorescent dye (DAPI) and visualized by DIC and fluorescence microscopy (Figure 1). The morphology of a typical wild-type zygotic ascus was slightly curved and contained four well-rounded spores, each with a single DAPI-staining body of equal intensity (Figure 1A). The *rec8*, *rec10*, and *rec11* mutants were proficient at meiosis and underwent two meiotic divisions as revealed by ascus development and the distribution of chromosomes (Figure 1, B–J). Strikingly, each mutant produced a high frequency of chromosome segregation errors that were sometimes accompanied by defects in ascus development (Figure 1, B–J). The data from a large number of asci from each mutant are presented schematically in Table 2.

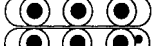
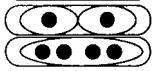
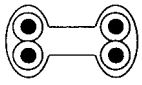
The cytological phenotypes of the *rec8* mutant were consistent with the reported (Molnar *et al.* 1995; Krawchuk and Wahls 1999) precocious separation of sister chromatids and nondisjunction of homologous chromosomes during MI: ascus development was nearly normal, but the chromosomes partitioned abnormally (Figure 1 and Table 2). In contrast, the chromosome segregation defects of the *rec10* and *rec11* mutants were often accompanied by additional defects such as abnormal spore placement, reduced spore numbers, and asynchronous spore maturation (Figure 1 and Table 2). These ascus phenotypes are characteristic of an MII defect because spore formation in *S. pombe* is controlled by the spindle pole body of the MII spindle (Tanaka and Hirata 1982; Shimoda *et al.* 1985; Hirata and Shimoda 1992, 1994). In some cases a defect in the segregation of sister chromatids during MII was directly inferred. For example, in Figure 1I it appears that the sister chromatids began to segregate from one another during MII and then became stuck. Thus, the $rec10^+$ and $rec11^+$ genes affect some function in MII in addition to their role in recombination during MI.

The *rec8*, *rec10*, and *rec11* mutants produce aneuploid meiotic products: In organisms with many chromosomes it is very difficult to study mutations affecting meiotic chromosome dynamics. Few products of such mutant meioses receive the appropriate number of chromosomes, and most of the meiotic products are aneuploid (of abnormal chromosome content) and inviable. Because *S. pombe* has only three pairs of chromosomes, aberrant segregation might produce relatively high frequencies of viable haploid and diploid missegregants if the chromosomes partition with some degree of randomness. This seems to be the case. The *rec8*, *rec10*, and *rec11* mutants produced 16, 66, and 79% viable meiotic products relative to wild-type cells (Figure 2A).

Random assortment of three chromosome pairs during one of the meiotic divisions should produce four classes of meiotic products: haploids, nullosomic aneuploids (missing one or more chromosomes), disomic aneuploids (having one or two additional chromosomes), and diploids. In *S. pombe* the haploids and diploids are viable, nullosomic aneuploids are inviable, and disomic aneuploids are unstable and rapidly lose the extra chromosome or chromosomes to become haploid (Niwa and Yanagida 1985). We therefore scored for the production of diploid spores with the understanding

TABLE 2

Cytological phenotypes of asci

		% of total in *rec*						
Class	Phenotype	+	8^-	10^-	11^-	8^-10^-	8^-11^-	10^-11^-
a (A)		98	30	58	53	35	32	55
b (B)		0.5	**28**	**5.5**	3.9	**19**	**20**	**6.0**
c		0	1.9	3.1	0	1.0	1.2	3.4
d (FG)		0	0	**5.5**	0.4	4.2	1.2	1.7
e		0	0.9	0.8	0	3.5	2.4	0.9
f (D)		0.5	**13**	2.4	0	**19**	16	0.9
g		0	3.2	0.8	0	2.6	3.5	0.4
h		0	0.9	0.4	0	0.3	0.4	0
i (C)		0.5	**16**	0	1.6	**7.1**	**13**	2.6
j		0	0	0	0	1.0	0	0
k (H)		0	0.5	0.8	**12**	3.2	1.6	6.9
l		0	0	1.2	0.4	0.6	0.4	0.4
m		0	0	0	0	0	0.4	0
n		0	4.6	3.1	4.7	2.9	5.1	4.7
o (I)		0	0	1.2	**9.1**	0	0.4	3.9
p		0	0	1.6	3.9	0	0.4	1.3
q (J)		0.5	1.4	2.8	**7.5**	0.3	0.4	**9.4**
r		0	0	0.8	3.1	0.6	0	0.4
s (E)		0	0	**12**	0.4	0.6	1.2	1.7
		n = 216	215	254	254	310	255	233

Randomly selected asci were classified based upon DNA content and distribution (black dots), spore coat formation (circles), and ascus morphology (peripheral oval) using data such as those in Figure 1. Class letters enclosed in parentheses correspond to the DAPI micrographs in Figure 1. The three predominant abnormal phenotypes for each mutant are shown in boldface type.

that this underestimates the frequency of missegregation events. Each of the *rec* mutants generated diploid spores at a frequency that was 10- to 50-fold higher than that from rec^+ crosses (Figure 2B).

Because *S. pombe* contains only three pairs of chromosomes, aberrant assortment during one or both of the meiotic divisions might produce some asci with aneuploid spores that, by chance, each contain a similar amount of DNA. We therefore dissected tetrads with normal morphology and we gauged the viability of each spore within each tetrad. The *rec8*, *rec10*, and *rec11* mutants each produced normal looking asci in which there was a high frequency of one or more inviable spores (Figure 2C). In each mutant the tetrad spore viability pattern was significantly different than that of wild-type cells. This indicates that the majority of the cytologically normal tetrads of the *rec8*, *rec10*, and *rec11* mutants were genetically abnormal.

In summary, in each mutant the severity of the meiotic chromosome segregation defects scored cytologically (Figure 1 and Table 2) was roughly proportional to the frequency of inviable meiotic products scored genetically (Figure 2A), to the frequency of meiotic diploid spores (Figure 2B), and to the frequency of cytologically normal, genetically abnormal asci (Figure 2C). Together, these data indicate that the majority of the *rec8*, *rec10*, and *rec11* mutant meioses were defective.

The $rec8^+$ gene is required for MI chromosome segregation, but $rec10^+$ and $rec11^+$ are required for segregation of sister chromatids during MII: Because the three mutants had aberrant chromosome segregation (Figure 1 and Table 2), produced a relatively high frequency of viable meiotic products (Figure 2A), and produced a relatively high frequency of meiotic diploids (Figure 2B), we were able to monitor directly the segregation patterns of individual chromosomes in the mutants. Meiotic diploids were genotyped for heteroallelic, centromere-linked genetic markers on each chromosome. Those data were compared to the various patterns of marker inheritance predicted to result from three different types of aberrant chromosome segregation (Figure 3, A–D).

The *rec8* mutants produced meiotic diploids that were predominantly heterozygous for each of the centromere-linked markers on chromosomes *I*, *II*, and *III* (Figure 3E). The average heterozygosity for loci on all three chromosomes was 75 ± 7%. We conclude that the $rec8^+$ gene is required mainly for the proper segregation of chromosomes during MI.

In contrast to the *rec8* mutants, the majority of the

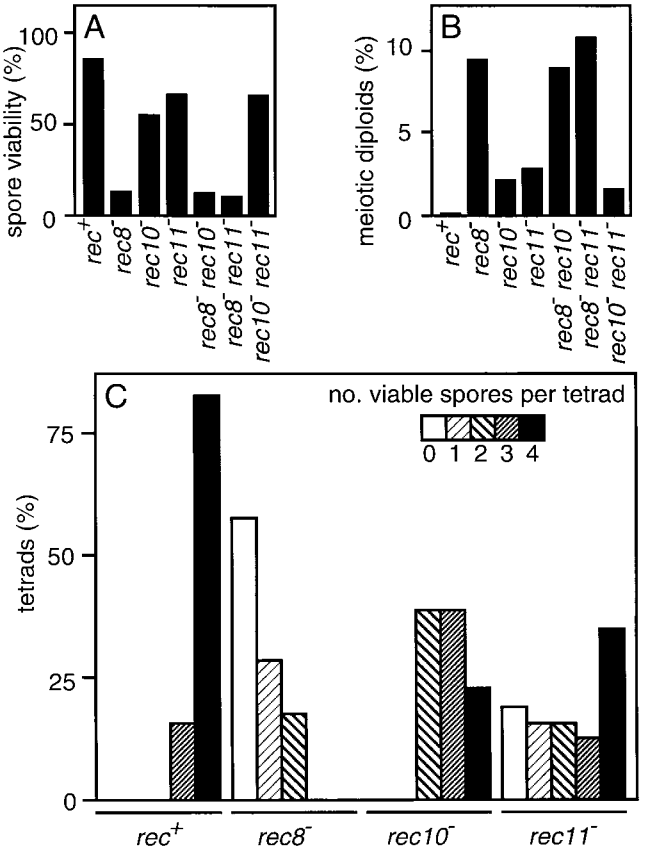


Figure 2.—Formation of aneuploid meiotic products. (A) Spore viabilities. (B) Frequencies of diploid meiotic products. Between 962 and 1538 spore colonies were genotyped for each value. (C) Spore viabilities in cytologically normal tetrads. Seventy-six tetrads with normal morphology were dissected and classified by the number of spores that were viable on nonselective medium. In each mutant the viability pattern is significantly different ($P \leq 0.05$) from that of wild type.

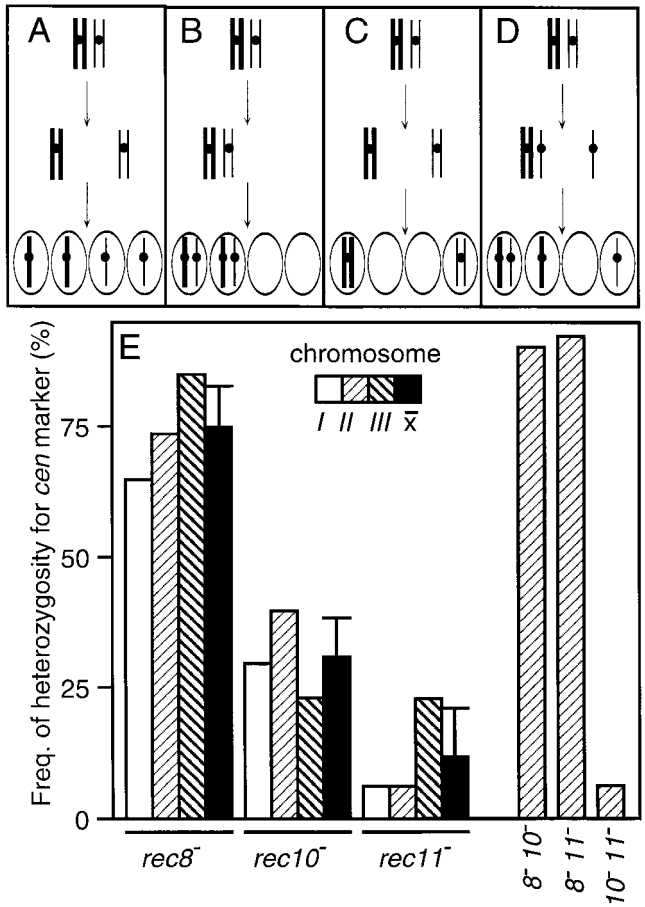


Figure 3.—Segregation patterns of chromosomes. (A) Normal meiosis produces four haploid products. The pattern by which centromeric markers are inherited reveals (B) MI nondisjunction, (C) MII nondisjunction, and (D) precocious separation of sister chromatids. If sister chromatids segregate at random during each of the meiotic divisions, 67% of diploid spores will be heterozygous for the centromere (Krawchuk and Wahls 1999). (E) Diploid spore colonies were genotyped for heteroallelic marker loci that were tightly linked to each of the three centromeres. The loci used were *lys1* (chromosome *I*), *tps13* (chromosome *II*), and *ade6* (chromosome *III*) and each value is based upon genotyping between 63 and 130 diploid spore colonies. For the analysis of double mutants, the *mat1* locus (chromosome *II*) was used and each value is based upon genotyping between 26 and 123 diploid spore colonies.

diploids produced by the *rec10* mutants were homozygous for each of the centromere-linked markers on chromosomes *I*, *II*, and *III* (Figure 3E). The average homozygosity for loci on all three chromosomes was 69 ± 7%. We conclude that the *rec10*$^+$ gene is required principally for the proper segregation of sister chromatids during MII.

The *rec11* mutants produced diploids that were predominantly homozygous for each of the centromere-linked markers on chromosomes *I*, *II*, and *III*, with an average homozygosity of 88 ± 8% (Figure 3E). We conclude that the *rec11*$^+$ gene is required mainly for the proper segregation of sister chromatids during MII.

The availability of centromere-linked markers allowed us to monitor simultaneously the segregation of each of the three chromosomes within individual cells (Table 3). The majority of the *rec8* mutants missegregated all three chromosomes during MI, and the majority of the *rec10* and *rec11* mutants suffered MII segregation errors for all three chromosomes. We conclude that the MI defect in the *rec8* mutants and the MII defects in the *rec10* and *rec11* mutants affect each of the three chromosomes within an individual cell with an approximately equal probability. Almost half of the individual diploids exhibited patterns characteristic of MI or MII missegregation for different chromosomes (Table 3). These segregation patterns provide additional evidence that two meiotic divisions had occurred and that the diploids resulted from meiotic chromosome segregation defects.

The *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes are required for recombination toward the centers of chromosomes: The *rec8*$^-$, *rec10*$^-$, and *rec11*$^-$ mutations were identified based upon a recombination defect at the *ade6* locus on chromosome *III* (Ponticelli and Smith 1989). Because these mutations affect the segregation of all three chromosomes (Figures 1–3; Tables 2 and 3), it seemed im-

TABLE 3

Aberrant segregation patterns of all three chromosomes within individual meiotic products

Type of segregation error on each chromosome[a]			Number of spores with segregation error		
Chromosome *I*	Chromosome *II*	Chromosome *III*	*rec8*$^-$	*rec10*$^-$	*rec11*$^-$
MI	MI	MI	26	0	4
MII	MI	MI	5	1	3
MI	MII	MI	1	2	1
MII	MII	MI	1	1	2
MI	MI	MII	4	2	4
MII	MI	MII	3	0	0
MI	MII	MII	1	6	5
MII	MII	MII	7	20	20

[a] The inferred type of segregation error for each chromosome within each diploid spore colony was determined by analysis of centromere-linked markers as in Figure 3E.

plausible that the recombination defect was restricted mainly to chromosome *III*, as previously reported (DeVeaux and Smith 1994). We therefore extended that study here and elsewhere (Krawchuk and Wahls 1999) to determine whether the *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes were required for crossover recombination at specific regions of all three chromosomes. The new data are presented in Table 4 and are summarized together with the published data in Figure 4. Intergenic (crossover) recombination near the center of chromosomes *I*, *II*, and *III* was compromised in the *rec8*, *rec10*, and *rec11* mutants. In contrast, recombination toward the ends of chromosomes *I* and *II* was nearly normal in the mutants. (It is not practical to measure intergenic recombination in the subtelomeric regions of chromosome *III* because those regions, which encompass about one-third of the chromosome, are occupied by the rDNA repeats.) In each mutant, and on each chromosome, the defects in recombination were similar and appeared to be a function of the physical distance from the approximate center of the chromosome. We conclude that the *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes are each required for recombination toward the centers of all three chromosomes during MI. This contradicts the previous claim (DeVeaux and Smith 1994) that *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ are chromosome *III*-specific activators of recombination.

The *rec8*$^+$ gene is epistatic to *rec10*$^+$ and to *rec11*$^+$: Because the *rec8*, *rec10*, and *rec11* mutants displayed some common phenotypes and some phenotypes that were distinct, we tested for genetic interactions between the three mutated genes. The *rec8 rec10* and *rec8 rec11* double mutants each produced the same predominant cytological phenotypes as the *rec8* single mutant (Table 2). Furthermore, in both cases the *rec8*$^-$ mutation suppressed the predominant mutant phenotypes conferred by the *rec10*$^-$ and *rec11*$^-$ mutations. These data demonstrate that *rec8*$^+$ is epistatic to both *rec10*$^+$ and *rec11*$^+$. Analyses of spore numbers in the asci (Table 2), spore viabilities (Figure 2A), frequencies of aneuploid meiotic products (Figure 2B), and patterns of chromosome missegregation for centromere-linked genetic markers (Figure 3E) in the single and double mutants each support this pathway assignment.

While *rec8*$^+$ was epistatic to *rec10*$^+$ and *rec11*$^+$, there was no clear epistatic relationship between *rec10*$^+$ and *rec11*$^+$. The *rec10 rec11* double mutant expressed cytological phenotypes that were distinct from those of the *rec10* and *rec11* single mutants, and the data were characteristic of an additive or intermediate phenotype (Table 2). However, other data suggest that a partial epistasis relationship may exist: There was no additivity for meiotic inviability (Figure 2A), or for the generation of meiotic diploidy (Figure 2B), or for the aberrant segregation pattern of a centromere-linked genetic marker (Figure 3E) in the *rec10 rec11* double mutant relative to the *rec10* and *rec11* single mutants. We conclude that *rec10*$^+$ and *rec11*$^+$ each participate in some distinct func-

TABLE 4

Intergenic (crossover) recombinant frequencies

	Recombinant frequency (%)[a]			
Genetic interval	Wild type	*rec8*$^-$	*rec10*$^-$	*rec11*$^-$
pro2-arg3	13	1.3	5.2	1.7
pro2-lys1	46	1.9	4.1	3.8
lys1-his1	19	10	6.1	3.8
lys1-leu2	58	4.7	30	5.5
arg6-his3	27	10	7.6	12
arg6-pat1	19	1.5	3.9	2.4
his3-pat1	23	1.7	ND[b]	0.77
leu1-his2	14	0.77	1.6	1.3
his5-leu3	47	12	6.5	6.0[c]

[a] Double prototrophic spore colony titer divided by viable spore colony titer × 200. At least 100 colonies of each type were counted for each frequency determination. Because diploid spore colonies might contain complementing markers, they were excluded from analysis (materials and methods).

[b] Not determined.

[c] This value is based on only 72 recombinant colonies.

tions, but there may be one or more additional functions in which the *rec10*$^+$ and *rec11*$^+$ genes participate together or share an epistasis relationship.

DISCUSSION

There are four principal findings of this study. First, the three *rec* genes have a partial epistasis relationship that places *rec8*$^+$ upstream of *rec10*$^+$ and *rec11*$^+$. Second, each of these three genes is required for a normal frequency and distribution of meiotic crossover recombination, in particular toward the centers of each of the three chromosomes. Third, the *rec8*$^+$ gene is required for proper chromosome segregation during MI, as reported (Molnar *et al.* 1995). And fourth, the *rec10*$^+$ and *rec11*$^+$ genes are required for the proper segregation of sister chromatids during MII.

An epistasis pathway of genes that are required for multiple meiotic functions: The *rec8*$^+$ gene is a member of a family of cohesin genes (Parisi *et al.* 1999) and is required for meiotic sister chromatid cohesion and homologous chromosome pairing (Molnar *et al.* 1995).

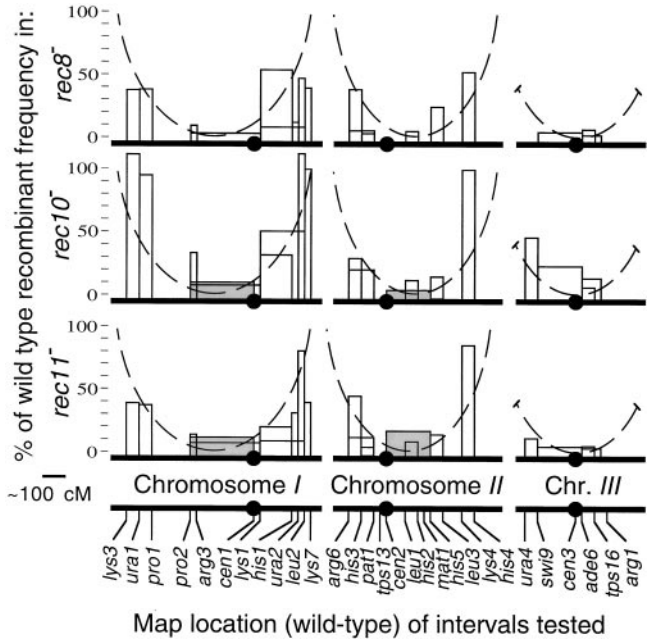


Figure 4.—Intergenic recombinant frequencies. The histogram bars indicate the approximate locations of the intergenic intervals tested on each chromosome and the frequency of crossing over within each interval relative to that in wild-type cells. The shaded bars indicate the upper limit to the frequency determined specifically in those meioses with aberrant chromosome segregation producing diploid spores. A curve plotting crossing over as a function of marker location (dashed line) was made for each chromosome and superimposed upon the data sets of each mutant for the sake of comparison. The rDNA repeats on the ends of chromosome *III* (about one-third of the chromosome length) are not shown on the schematic. The data are from this study (Table 4), DeVeaux and Smith (1994), and Krawchuk and Wahls (1999) and wild-type genetic map coordinates are those of Munz (1994).

Because *rec8*$^+$ is epistatic to both *rec10*$^+$ and *rec11*$^+$ (Table 2; Figures 2 and 3), and the *rec10*$^+$ and *rec11*$^+$ genes are each required for the proper segregation of sister chromatids during MII (Figure 3), we propose that these genes define a pathway of "meiotic chromatid cohesion."

The lack of clear genetic interactions between *rec10*$^+$ and *rec11*$^+$ indicates that the inferred pathway is not simply linear. The presence of shared (Figures 2–4; Tables 3 and 4) and distinct (Figures 1 and 2; Table 2) phenotypes in the single and double mutants suggests that *rec10*$^+$ and *rec11*$^+$ might function together for some processes, but function independently for others.

rec10$^+$ and *rec11*$^+$ are required for recombination (Table 4; Figure 4) and must therefore have some function in meiotic prophase or MI. Intriguingly, the *rec10* and *rec11* mutants exhibit mainly MII segregation errors (Table 3; Figure 3). This is paradoxical because hyporecombination mutants should affect MI segregation (Roeder 1997; Moore and Orr-Weaver 1998). However, the paradigm is from organisms in which MI defects activate a checkpoint or produce inviable meiotic products, precluding genetic identification of potential MII phenotypes.

S. pombe is different in two regards. First, the recombination defects in the *rec8*, *rec10*, and *rec11* mutants do not confer a checkpoint arrest or barrier to development; meiosis proceeds and meiotic products are formed (Figure 1). Second, because *S. pombe* contains only three pairs of chromosomes, abnormal or random assortment can produce a relatively high frequency of meiotic products that receive at least one copy of each chromosome and are therefore viable (Figure 2). The high viability of meiotic products from the *S. pombe rec10* and *rec11* mutants allowed us to visualize the MII errors (Figure 3).

A model of chromosome pairing dependent upon Rec8, Rec10, and Rec11: In fission yeast, as in many organisms, telomeres become clustered during meiotic prophase to form a "bouquet" structure prior to pairing of other regions of chromosomes (Chikashige *et al.* 1994, 1997; Shimanuki *et al.* 1997; Cooper *et al.* 1998; Nimmo *et al.* 1998). The available data suggest a model in which the Rec8, Rec10, and Rec11 proteins are required for subsequent pairing of internal chromosome regions (Figure 5).

After telomere clustering (Figure 5A), migration of the spindle pole body leads the clustered telomeres back and forth and the unpaired central regions of the chromosomes trail behind in a structure called a "horsetail" (Figure 5, B–C; Kohli 1994; de Lange 1998). The net effect is that the internal regions of the chromosomes of similar length fall into rough alignment (Figure 5C). This may facilitate local searches for homology or there may simply be fortuitous, chance encounters where homologous DNA sequences come into close contact with one another. We suggest that the Rec10 and Rec11 proteins, like Rec8 (Molnar *et al.* 1995), are

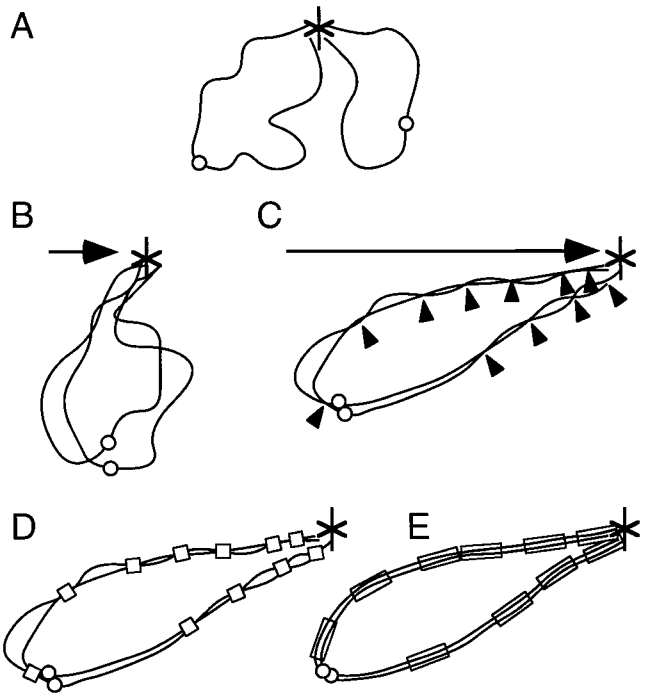


Figure 5.—A model of homologous chromosome pairing. Lines represent homologous chromosomes (a pair of chromatids) and circles represent the centromeres. (A) Telomeres cluster and associate with the spindle pole body (asterisk) to form the bouquet structure. (B) The telomeres are pulled back and forth by the spindle pole body. (C) This results in a rough alignment of individual chromosomes with multiple interstitial points of contact between homologous sequences (triangles). (D) Components of the meiotic chromatid cohesion pathway stabilize these interstitial contacts and promote further pairing between nearby regions of the homologous chromosomes (squares). (E) Discontinuous patches of linear elements (rectangles) form to complete synapsis. In the absence of full pairing and synapsis, recombination can still occur at the multiple interstitial interactions between homologous DNA sequences (C), which are much more abundant near the telomeres due to the telomere clustering.

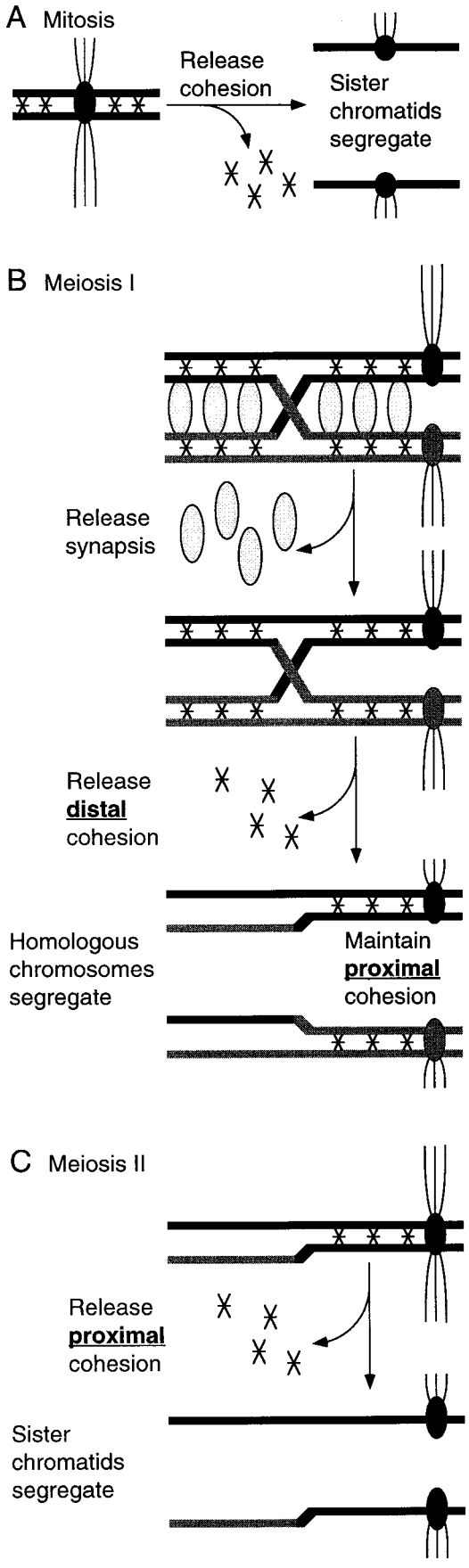
required to stabilize the interstitial points of contact between the homologous chromosomes (Figure 5D) and to promote the pairing of regions in the vicinity (Figure 5E). While pairing has not been directly studied in the *rec10* and *rec11* mutants, the similar regional recombination defects in each of the *rec8*, *rec10*, and *rec11* mutants (Figure 4) may simply be a consequence of defects in pairing: Recombination could still occur at a high rate near the telomeres, where homologous chromosomes are in close proximity due to telomere clustering, but might be severely compromised at the more central regions, where homologs are not properly paired (Figure 5C).

An alternative (not mutually exclusive) possibility is that the Rec8, Rec10, and Rec11 proteins have a more direct role in distributing recombination events along chromosomes. For example, certain recombination enzymes may serve some active function in the pairing process, such as testing the homologous register of the interstitial points of contact between chromosomes (Figure 5, D and E). As another example, the Rec8, Rec10, or Rec11 proteins might nucleate the assembly of recombination enzymes to recombination nodules. It will be informative to determine whether these proteins and known enzymes of recombination colocalize with each other.

Functions for chromatid cohesion in both MI and MII segregation: In most organisms chiasmata have a role in maintaining the paired state between homologous chromosomes prior to anaphase of MI. However, in the absence of some type of additional glue, chiasmata will migrate off the bivalent and the homologs will separate. Darlington (1932) and others (*e.g.*, Maguire 1974) suggested that a cohesive interaction between sister chromatids distal to crossovers would stabilize the chiasmata to prevent their loss (reviewed by Moore and Orr-Weaver 1998).

A model for how crossovers and sister chromatid cohesion relate to meiotic chromosome segregation is depicted in Figure 6. Crossovers and cohesion distal to the point of exchange work synergistically to hold bivalents together. The distal cohesion must be dissolved during MI so that the homologs can segregate. However, cohesion at or near to the centromere (proximal to the point of exchange) must be maintained during MI to ensure that sister chromatids segregate to the same pole and to hold the sister chromatids together on the metaphase plate of MII while under spindle tension. During anaphase of MII, this centromere-proximal cohesion must be properly released to allow sister chromatids to segregate.

A hypothesis about Rec8 and the influence of crossover location on the stability of interhomolog cohesion during MI: Fluorescence *in situ* hybridization of wild-type *S. pombe* meioses revealed that sister chromatids are normally in close proximity to one another, whereas in 20% of *rec8* mutant meioses the sister chromatids are widely separated (Molnar *et al.* 1995). Also in that study, genetic analysis of chromosome *III* disomy patterns in tetrads demonstrated that 4.4% (3 of 68) of *rec8* mutant meioses suffered precocious separation of one pair of chromosome *III* sister chromatids. However, as pointed out by the authors, the analysis of chromosome *III* disomics would have missed MI nondisjunction events involving more than just chromosome *III*, and the assay system precluded the identification of any MII nondisjunction events. Our approach to study segregation errors on all three chromosomes (Figure 3; Table 3) also has some limitations. In particular, we cannot distinguish between MI segregation errors due to nondisjunction and those due to precocious separation of sister chromatids. Because chromatid cohesion defects clearly occur but do not seem to affect all *rec8* mutant meioses (Molnar *et al.* 1995), and chromatids of homologous chromosomes can recombine to (presumably)



generate chiasmata (Table 4; Figure 4), nondisjunction may contribute to the MI segregation errors. This seems to be the case. Novel centromere mapping functions for aneuploid meiotic products revealed significant levels of MI nondisjunction in the *rec8* mutants (Krawchuk and Wahls 1999).

There are between 11 and 17 crossovers per chromosome in a wild-type *S. pombe* meiosis (Munz 1994) and reciprocal recombination in distal regions of chromosomes of *rec8* mutants is nearly normal (Figure 4), so at least one crossover is statistically present on each chromosome in the majority of the mutant meioses. And yet, the majority of those meioses suffer chromosome segregation errors (Figures 1–3; Tables 2 and 3). Thus, it appears that the residual crossovers are insufficient to ensure proper MI segregation in the *rec8* mutants.

Some MI segregation errors in the *rec8* mutants might be the result of either the decreased frequency or the abnormal distribution of crossovers. However, either of these seems unlikely to be the sole causative factor because the *rec10* and *rec11* mutants have a similar frequency and distribution of crossovers as the *rec8* mutants (Figure 4) but do not suffer predominantly MI segregation errors (Figure 3). Intriguingly, chromosomes that have suffered spontaneous MI nondisjunction in Drosophila, budding yeast, and humans exhibit a preferential reduction in centromere-proximal crossovers (Gethmann 1984; Rasooly *et al.* 1991; MacDonald *et al.* 1994; Moore *et al.* 1994; Sherman *et al.* 1994; Hassold *et al.* 1995). This parallel to the recombination and segregation phenotypes of the *rec8* mutants suggests that some combination of sister chromatid cohesion and crossover position is important for MI chromosome segregation.

The patches of discontinuous linear elements on synapsed *S. pombe* chromosomes, thought to be related to axial elements of the SC in other organisms, break down before the two meiotic divisions (Bähler *et al.* 1993; Kohli 1994; Kohli and Bahler 1994; Scherthan *et al.* 1994). Thus, the structural scaffold supporting synaptic

Figure 6.—Regulated dissolution of cohesive forces. The lines represent individual chromatids. (A) Mitotic cohesion (asterisks) holds sister chromatids together in opposition to spindle tension. A complete release of cohesion allows the chromatids to segregate during mitosis. (B) Meiotic cohesion (asterisks) provides cohesive forces between sister chromatids and contributes to pairing forces between homologs. A release of synapsis is insufficient to permit the homologous chromosomes to segregate, because they remain firmly attached to each other via the sister chromatid cohesion distal to the crossover (crossed chromatids). A release of chromatid cohesion distal to the crossover allows the homologous chromosomes to segregate from one another during MI. (C) Cohesion proximal to the crossover, at least at the centromere, must be maintained to keep sister chromatids associated and to oppose spindle forces as each chromosome subsequently aligns on the metaphase plate of MII. The second meiotic division then proceeds with a complete release of cohesion.

interhomolog interactions is probably disassembled prior to the two meiotic divisions. This leaves in place the sister chromatid cohesion interactions, which, when accompanied by crossovers, may be sufficient to hold together both pairs of sister chromatids and the pair of homologous chromosomes (Figure 6B). An attractive hypothesis is that the centromere-proximal crossovers are more proficient at ensuring proper MI chromosome segregation than distal crossovers because the proximal crossovers leave a greater extent of interhomolog sister chromatid cohesion than distal crossovers (Figure 6B). If the crossover is too close to the telomere or if the distal cohesion is defective, the interhomolog cohesive force would be inadequate to oppose the repeated, register-testing forces of the spindle apparatus. Once torn apart, the homologous chromosomes cannot form the proper bipolar spindle attachment and therefore segregate independently. By chance, half of the time they will segregate to different poles, and half of the time they will arrive at the same pole, thus giving rise to an MI nondisjunction phenotype.

A hypothesis about Rec10, Rec11, and the influence of crossover location on the stability of intersister cohesion during MII: The *rec10*$^+$ and *rec11*$^+$ genes are required for the proper segregation of sister chromatids during MII (Figure 3; Krawchuk and Wahls 1999). Mutations affecting MII chromatid cohesion and segregation are quite rare, but they have been described in a few organisms (Clayberg 1959; Davis 1971). As one well-characterized example, the *mei-S332* mutants of Drosophila fail to maintain cohesion between sister chromatids during or just after anaphase of the MI division (Kerrebrock *et al.* 1992). Furthermore, the mei-S332 protein localizes to centromeric regions and dissociates at the onset of MII anaphase, when sister chromatids segregate from one another (Kerrebrock *et al.* 1995; Moore *et al.* 1998). It will be of interest to determine whether the Rec10 and Rec11 proteins behave in a similar fashion.

An elevated frequency of centromere-proximal crossing over has been found on chromosomes that suffered spontaneous MII nondisjunction in humans and Drosophila, suggesting that crossover position can also affect the segregation of sister chromatids during MII (MacDonald *et al.* 1994; Fisher *et al.* 1995; Lamb *et al.* 1996; Koehler *et al.* 1996a,b). The *rec10* and *rec11* mutants exhibit MII segregation errors (Figure 3) but have a reduced frequency of centromere-proximal crossing over (Figure 4; Krawchuk and Wahls 1999). We suggest that Rec10 and Rec11 are required for proper centromere-proximal sister chromatid cohesion during MII and that their absence recapitulates the effect of placing a crossover too close to the centromere. In each case, the intersister cohesive force would be inadequate to oppose the repeated, register-testing forces of the MII spindle apparatus (Figure 6C). Once torn apart, the sister chromatids would segregate independently. By chance, half of the time they will segregate to different poles, and half of the time they will arrive at the same pole, thus giving rise to an MII nondisjunction phenotype.

The machinery of the reductional division is sufficiently intact in the *rec10* and *rec11* mutant meioses to ensure proper segregation of homologs during MI most of the time. However, the *rec10* and *rec11* mutants do experience 31 and 12% of MI segregation errors, respectively (Figure 3). Whether these MI segregation errors are a consequence of homologous chromosome pairing defects, of defects in the overall crossover frequency, of defects in the distribution of crossovers, or of defects in sister chromatid cohesion is a matter for conjecture.

Summary: Analysis of the *rec8*, *rec10*, and *rec11* mutants revealed a "meiotic chromatid cohesion" pathway that links together sister chromatid cohesion, pairing of internal regions of homologous chromosomes, centromere proximal recombination, and the proper segregation of chromosomes during each of the meiotic divisions. Additional functions might include the preferential selection of homologs (rather than sister chromatids) as partners for recombination, stabilization of chiasmata (crossovers), and ensuring that the additional kinetochores are shielded during MI and presented during MII for proper spindle attachment.

We thank Charlie Albright, Mari Davidson, Aaron Graff, Jürg Kohli, Ning Kon, Michael Lichten, Gisela Mosig, Wallace Sharif, and Gerry Smith for helpful discussions; Calley Hardin and Steve Lindsey for laboratory assistance; Kathy Gould, Nancy Hollingsworth, Jürg Kohli, and Gerry Smith for yeast strains; and Jürg Kohli for sharing results prior to publication. This work was supported by a grant from the National Institutes of Health (GM54671) and pilot project funds from the Vanderbilt University Research Council. M.D.K. was supported in part by a training grant from the National Institutes of Health (CA09582), L.C.D. was supported by a grant from the National Institutes of Health (GM31693), and W.P.W. was a Leukemia Society of America Special Fellow (3021-94) for a portion of this research.

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Communicating editor: M. Lichten