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The Yeast *med1* Mutant Undergoes Both Meiotic Homolog Nondisjunction and Precocious Separation of Sister Chromatids

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ABSTRACT

A mutant at the yeast *MED1* locus was isolated in a screen for sporulation-proficient, meiotic-lethal mutants. Synaptonemal complex formation in the *med1* mutant is apparently normal and *med1* strains undergo meiotic crossing over at approximately 50% of the wild-type level. The *med1* mutant undergoes homolog nondisjunction at meiosis I, presumably as a consequence of the decrease in crossing over. In addition, the mutant undergoes precocious separation of sister chromatids, resulting in chromosome missegregation at both meiotic divisions. We suggest that the *med1* mutation perturbs chromosome structure, leading to a reduction in recombination and a defect in sister chromatid cohesion.

MEIOSIS is the mechanism whereby diploid organisms reduce their chromosomal content precisely in half in order to benefit from sexual reproduction. During meiosis, two rounds of chromosome segregation follow a single round of chromosome replication. The first round of chromosomal segregation, in which homologous chromosomes (homologs) segregate from each other, is unique to meiosis. There are two requirements for a proper reductional division. First, homologous chromosomes must locate each other and pair to form a bivalent. Second, the homologs must remain associated in some way until anaphase I.

In mitosis, sister chromatid cohesion holds chromatids together until anaphase. Sister chromatid cohesion is thought to be due, at least in part, to the residual intertwinings of the DNA helices resulting from DNA synthesis. Consistent with this hypothesis, topoisomerase II is required for mitotic anaphase (HOLM *et al.* 1985; UEMURA and YANAGIDA 1986). Alternatively, or in addition, sister chromatids might be held together specifically in the kinetochore region. Mitotic chromosomes are stabilized at the metaphase plate by the connections between sister chromatids and the connections between kinetochores and spindle poles (NICKLAS 1974). The tension applied to the chromatids by microtubules from opposite spindle poles is resisted by sister chromatid cohesion, which prevents segregation until anaphase.

For paired chromosomes to align properly at the metaphase plate in meiosis, tension must exist between the chromosomes and the spindle poles, as in mitosis (NICKLAS 1974). In meiosis, genetic crossovers establish connections between homologs that permit them to behave as a unit at metaphase I. These connections, called chiasmata, can be observed cytologically in some organisms and they persist until the chromosomes segregate (JONES 1987). Although crossovers are essential for reductional segregation, they are not sufficient. Analyses of meiotic mutants in maize, Drosophila and yeast have demonstrated that recombinant chromosomes can nondisjoin, suggesting that the connections are not maintained in these mutants (HAWLEY 1988; ROCKMILL and FOGEL 1988; ROCKMILL and ROEDER 1990; ENGEBRECHT, HIRSCH and ROEDER 1990; FLATTERS and DAWSON 1993). Furthermore, the sequence context in which recombination takes place also determines whether a crossover ensures meiotic disjunction. In artificial chromosomes (MURRAY and SZOSTAK 1983) crossovers in yeast DNA sequences ensure proper meiosis I disjunction, whereas crossovers in bacteriophage lambda DNA do not (ROSS *et al.* 1992).

The synaptonemal complex (SC) is the proteinaceous structure that is apparent when chromosomes are synapsed (VON WETTSTEIN, Rasmussen and HOLM 1984). Recombination nodules are cytologically defined spheres or ellipsoids found along SCs with the same frequency and distribution as recombination events (CARPENTER 1988). Chiasmata are thought to contain remnants of both the SC and recombination nodules (HOLM and RASMUSSEN 1980). It is possible that the bivalent is held together by these proteins (chiasma binders). Alternatively, sister chromatid cohesion distal to chiasmata could hold homologs together. From cytological studies of maize, MAGUIRE (1990) has concluded that the latter possibility is probably the case. It is also possible that both mechanisms function in meiosis.

In this paper, the yeast *med1* mutant is described.

TABLE 1

Yeast strains

Strain	Genotype
BR2754-1C	*MAT***a** *leu2-27 thr1-4 trp1-289 ura3-1 ade2-1 adex med1-1*
BR2754-4B	*MATα leu2-27 his4-260 arg4-8 thr1-4 trp1-289 ura3-1 ade2-1 med1-1*
BR2774-BR2776	*MATα leu2-3, 112 his4-260 ura3-1 trp1-289 lys2 spo13::ura3-1 ARG4 thr1-4 ade2-1 med1-1* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 lys2spo13::ura3-1 arg4-8 thr1-1 ade2-1 med1-1*
BR2777-BR2779	Congenic with BR2774, except *MED1*/*med1-1*
BR2780-BR2782	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 lys2spo13::ura3-1 ARG4 thr1-4 ade2-1 med1-1* *MAT***a** *leu2-27 his4-260 ura3-1 trp1-289 lys2spo13:ura3-1 arg4-8 thr1-1 ade2-1 med1-1*
BR2783-BR2785	Congenic with BR2780, except *MED1*/*med1-1*
BR2800	BR2774, except *mek1::LYS2*/*mek1::LYS2*
BR2801	BR2777, except *mek1::LYS2*/*mek1::LYS2*
BR2804-BR2808	*MATα leu2-27 HIS4 ura3-1 ade2-1ADE6 CYH3 TRP5 met13spo13::ura3-1 arg4-8 thr1-4 MED1* *MAT***a** *LEU2 his4-260 ura3-1 ade2-1 ade6 cyh3 trp5 MET13 spo13::ura3-1 arg4-8 thr1-1 med1-1*
BR2809-BR2810	Congenic with BR2804, except *med1-1*/*med1-1*
BR2821	BR2774, except *mer1::LYS2*/*mer1::LYS2*
BR2822	BR2777, except *mer1::LYS2*/*mer1::LYS2*
BR2819	BR2774, except *mei4::URA3*/*mei4::URA3*
BR2820	BR2777, except *mei4::URA3*/*mei4::URA3*
BR2837	*MATα leu2-27 his4-280 ura3-1 ADE2 TRP1 arg4-8 thr1-1* $CUP1^S$ *med1-1* *MAT***a** *leu2-27 his4-280 ura3-1 ade2-1 trp1-1 arg4-9 THR1* $CUP1^S$ *med1-1*
BR2877	*MAT***a** *CENIII::TRP1 leu2::arg4-8* $CUP1^S$ *HIS4 trp1 ura3 ade2 arg4-Δ cup1-Δ med1-1* *MATα CENIII::URA3 leu2::arg4-8* $CUP1^S$ *his4 trp1 ura3 ade2 arg4-Δ cup1-Δ med1-1*

The *med1* mutant (*me*iotic *d*isjunction) is defective in meiotic chromosome segregation, yet is proficient in chromosome synapsis and undergoes nearly wild-type levels of crossing over. We suggest that the *MED1* gene product modulates chromosome structure and thereby indirectly affects sister chromatid cohesion and genetic recombination.

MATERIALS AND METHODS

Media, genetic techniques and strain constructions: Media and most genetic techniques have been described by SHERMAN, FINK and HICKS (1986). Copper medium and sporulation medium were described by ROCKMILL and FOGEL (1988). Yeast strains are listed in Table 1.

The original isolate of *med1-1*, a homothallic diploid, was sporulated and backcrossed at least seven times to wild-type laboratory strains to construct the strains used in this study. Congenic diploids for the recombination assays were made by mating a mutant haploid to three mutant and three wild-type segregants from the same cross. Two sets of strains, each consisting of three wild types and three mutants, were used for the measurement of meiotic prototroph frequencies. Another set of four wild-type and two mutant strains was used for the crossover analysis. No variation in phenotype was seen among genotypically equivalent strains; therefore, the data from congenic strains were pooled. Other meiotic mutant derivatives were constructed by transforming the haploid parents that make up a pair of mutant and wild-type congenic diploids (BR2774 and BR2777) with the appropriate plasmids and mating the resulting transformants. The following plasmids were used to disrupt meiotic genes: pB121, *mek1::LYS2* (ROCKMILL and ROEDER 1991); pTM6, *mei4::URA3* (MENEES and ROEDER 1989); pME6, *mer1::LYS2* (ENGEBRECHT and ROEDER 1989).

Complementation tests were carried out by transforming BR2837 with plasmids carrying wild-type meiotic genes and assaying spore viability by tetrad dissection. The following plasmids were used for complementation tests: pB64 (*RED1*) (ROCKMILL and ROEDER 1988); pME1 (*MER1*) (ENGEBRECHT and ROEDER 1989); A62-3 (*HOP1*) (HOLLINGSWORTH, GOETSCH and BYERS 1990); pB107 (*MEK1*) (ROCKMILL and ROEDER 1991); pMB47 (*ZIP1*) (SYM, ENGEBRECHT and ROEDER 1993); pJB45 (*SPO11*) (J. BHARGAVA, unpublished data); pTM12 (*MEI4*) (MENEES and ROEDER 1989); pME56 (*MER2*) (ENGEBRECHT, HIRSCH and ROEDER 1990); pJB1 (*REC102*) (BHARGAVA, ENGEBRECHT and ROEDER 1991); pRM212 (*REC104*) (GALBRAITH and MALONE 1992); pDP27.1 (*REC114*) (R. MALONE, unpublished data).

Strain BR2877 was constructed through a series of crosses

involving 6DLeu (SPECTOR and FOGEL 1992), which contains deletions of *ARG4* and *CUP1* on chromosome *VIII* and an insertion of these sequences at the *LEU2* locus. Insertion of the *URA3* gene at *CENIII* was achieved by transformation with plasmid pJC3-13 (CLARKE and CARBON 1981). Insertion of the *TRP1* gene on chromosome *III* was accomplished by transformation with pB19 made by CLAIRE MOORE. In this plasmid, a 1900-bp *Eco*RI-*Sau*3A fragment adjacent to *CENIII* replaces the centromere-containing *Eco*RI-*Bgl*II fragment in pUN10 (ELLEDGE and DAVIS 1988). pB19 was digested with *Cla*I to target integration at *CENIII*, resulting in a duplication of *CENIII*-adjacent sequences with vector DNA and *TRP1* inserted between the repeats.

Isolation of copper-resistant spores: Copper-resistant spores were isolated in two ways. In method I, diploids were sporulated in liquid medium and then either undisrupted asci or physically isolated spores (ROCKMILL, LAMBIE and ROEDER 1991) were plated on rich medium. The resulting colonies were replica-plated to copper-containing medium. For method II, patches of cells were sporulated on solid medium and then replica-plated to copper-containing medium. After 3 days, copper-resistant papillae were picked and patched onto rich medium.

Measurement of chromosome loss: Wild-type and *med1-1* homozygous diploid strains were grown to saturation and mated to an excess of a mating-competent haploid strain in fresh medium. After 5-hr incubation, aliquots were plated on selective medium to detect successful matings. Three cultures of each strain were mated, and the median frequencies of maters are reported.

Scoring of recombination in disomic spores: The *arg4-8* mutant is temperature-sensitive, capable of growth without arginine at or below 30°. *arg4-8* and *arg4-9* display intragenic complementation; the heteroallelic diploid can grow on medium lacking arginine at temperatures at or below 36°. Disomic spores derived from BR2837 were considered recombinant in the *ARG4-THR1* interval if they displayed the following phenotypes (genotypes in parentheses): Arg$^+$ at temperatures up to 36°, Thr$^-$ (*arg4-8/arg4-9 thr1/thr1*); Arg$^+$ at temperatures up to 30°, Thr$^+$ (*arg4-8/arg4-8 thr1/THR1*). The number of the first type was doubled to account for the presumed equal frequency of *arg4-8/arg4-9 THR1/THR1* disomes. The mating-competent Arg$^-$ disomes (*arg4-9/arg4-9*) were crossed to a *thr1* tester strain, and the resulting diploids were sporulated and tetrads were dissected to detect the recombinant *THR1/thr1* disomes. The number of these was corrected for those that did not mate and then added to the recombinant pool. The map distance in the disomes was calculated as the number of recombinant chromatids multiplied by 100 and then divided by the total number of chromatids.

Recombination on chromosome *III* in strain BR2877 was scored between *CENIII* and *MAT*. Disomic spores that were Ura$^+$ Trp$^+$ were scored as recombinant if they were mating competent. Ura$^+$ Trp$^-$ disomes were scored as single recombinants if they were nonmaters and as double recombinants if they were **a**-maters. Ura$^-$ Trp$^+$ disomes were scored as recombinant if they were nonmaters and as double recombinants if they were α-maters. Map distance was calculated by multiplying the frequency of recombinant chromatids by 100.

Cytology: Meiotic nuclei were spread according to the protocol of DRESSER and GIROUX (1988). To observe chromosomes at the light microscope, cells prepared according to the method of DRESSER and GIROUX (1988) were spread onto poly-L-lysine coated slides. To coat slides, a solution of 0.5 mg/ml poly-L-lysine (Sigma) was pipetted onto clean glass slides; after 15-min incubation the slides were rinsed with water and air dried. The chromosomes were stained with 1 mM DAPI (4′, 6-diamidino-2-phenyl-indole) for 30 min, rinsed with water and mounted with 70% glycerol, 2% n-propyl gallate.

TABLE 2

Spore viability in *med1* strains

Strain	Relevant genotype	No. of spores	% spore viability
	MED1 / *med1* [a]	216	95
	med1 / *med1* [a]	204	20
BR2777	*MED1 spo13* / *med1 spo13*	104	72
BR2774	*med1 spo13* / *med1 spo13*	104	71
BR2820	*MED1 mei4 spo13* / *med1 mei4 spo13*	104	84
BR2819	*med1 mei4 spo13* / *med1 mei4 spo13*	104	88

[a] Strains for this analysis were diploids resulting from matings between the haploid parents of diploids BR2774-BR2785 and either BR2754-1C or BR2754-4B.

RESULTS

***med1* is a meiotic-lethal mutant:** The *med1-1* mutant was isolated in a screen for meiotic-lethal mutants. Spores from a homothallic strain were mutagenized with ultraviolet light, and the resulting colonies were sporulated and assayed for spore viability by an ether test, as described previously (ROCKMILL and ROEDER 1988; ROCKMILL, LAMBIE and ROEDER 1991). Preliminary characterization of the *med1-1* mutant revealed that it produces 20% viable spores. The *med1-1* mutation is recessive and segregates as a single Mendelian character as assayed by spore viability. After repeated backcrosses to wild-type laboratory strains, spore viability was reassayed. Twenty percent of the spores were viable (Table 2) and many of the viable spores originated from four-spore viable tetrads (6% 4-spore viable tetrads, 6% 3-spore viable tetrads, 14% 2-spore viable tetrads and 10% 1-spore viable tetrads).

No mitotic defects in growth, mating, recombination or sensitivity to methylmethane sulfonate have been observed (data not shown). The frequency of chromosome loss was measured by a quantitative mating test (DUTCHER and HARTWELL 1982). In a *MAT***a**/*MAT*α diploid, mitotic loss of chromosome *III* leads to a mating-competent cell. *med1-1* homozygous diploids mate no more frequently than the corresponding wild type (3.1×10^{-7} and 5.0×10^{-7}, respectively).

All the experiments in this paper were performed with the UV-induced *med1-1* allele, which will be referred to throughout the remainder of this paper simply as *med1*. It is not known whether this mutation results in a complete loss of *MED1* function.

Spore inviability in the *med1* mutant is due to chromosome missegregation: To determine whether the spore inviability in *med1* strains is due to the missegregation of chromosomes, spores were isolated (ROCKMILL, LAMBIE and ROEDER 1991) and analyzed for the presence of extra chromosomes. Nonmating spores carry two copies of chromosome *III* and copper-resistant spores carry two copies of chromosome *VIII*. In a population of random spores from a *med1* homozygous diploid (BR2837), 4.3% were nonmaters (11/256) and 6.6% were copper-resistant (17/256). Meiotic chromosome missegregation in wild type is less than 0.01%. The relatively low level of disomy in the *med1* homozygote compared to other meiotic mutants (*e.g.*, *red1*; Rockmill and Roeder 1988) probably reflects the relatively high level of spore viability and especially the four-spore viable tetrads in which all chromosomes segregated properly (30% of the viable spores are from 4-spore viable tetrads).

Missegregation of chromosomes can occur in three ways (Figure 1): (1) both members of a homolog segregate to the same pole at meiosis I (type IA); (2) one intact chromosome pair and one sister of the homologous chromosome segregate to the same pole at meiosis I (type IB); or (3) both chromatids of one chromosome segregate to the same pole at meiosis II (type II). Because there is relatively high spore viability in the *med1* mutant and many tetrads contain two or three viable spores, it is possible to identify the different types of chromosome missegregation by tetrad analysis.

Two different *med1* homozygous diploids were analyzed by tetrad dissection in order to detect chromosome missegregations. Tetrads from strain BR2837 were analyzed to identify spores disomic for chromosome *III* or *VIII*, as described above. The heterozygous, centromere-linked marker, *TRP1*, on chromosome *IV* was used to distinguish sister spores. Additional tetrads were dissected from BR2877 in which *CUP1* (and *arg4-8*) are present on chromosome *III* instead of chromosome *VIII* (Figure 2). Chromosome *III* disomes derived from this strain are copper-resistant. Meiosis I and meiosis II nondisjunctants can be distinguished because the chromosome *III* centromeres are differentially marked with *URA3* or *TRP1*. Chromosome *III* disomes resulting from missegregation at meiosis I are Ura^+ Trp^+; disomes resulting from nondisjunction at meiosis II are either Ura^+ Trp^- or Ura^- Trp^+. The results of tetrad analysis (Table 3) indicate that all three types of chromosome missegregation occur in the *med1* homozygote.

***med1* spore inviability is relieved by a *spo13* mutation:** In meiosis, *spo13* mutants undergo a single round of predominantly equational chromosome segregation to form dyads containing diploid spores (KLAPHOLZ and ESPOSITO 1980). Many meiotic-lethal mutants that are recombination-deficient form viable spores in a *spo13* background (PETES, MALONE and SYMINGTON 1991), because recombination is not required for equational segregation. The spore viability of a *med1 spo13* double mutant is equivalent to that of a *spo13* single mutant (Table 2), suggesting that *MED1* function is required for the reductional division.

Recombination in *med1* strains: Most previously described meiotic-lethal mutants of yeast display moderate to severe defects in meiotic recombination. Sets of *med1 spo13* and *MED1 spo13* congenic strains were constructed to compare crossing over and gene conversion frequencies. Crossing over was measured in five intervals on two chromosomes. Map distances in *med1* strains were only 1.5- to 2.2-fold lower than the corresponding wild-type values (Table 4). Gene conversion was measured by determining the frequencies of prototrophs at four heteroallelic loci. Meiotic conversion in *med1* strains was reduced 1.4- to 5.9-fold compared with the corresponding wild-type strains (Table 5).

spo13 diploids have reduced spore viability compared to wild-type strains, presumably because crossovers interfere with equational chromosome segregation (KLAPHOLZ and ESPOSITO 1980). When *spo13* strains are made recombination-defective (*e.g.*, by a mutation in *rad50* or *mei4*), spore viability increases to approximately *SPO13* levels (MALONE and ESPOSITO 1981; MENEES and ROEDER 1989). Spore viability in both the *MED1 spo13* and *med1 spo13* strains increased when the *MEI4* gene was disrupted (Table 2). Thus, recombination interferes with equational chromosome segregation in both *med1 spo13* and *MED1 spo13* strains.

***MED1* is not a previously described gene:** The *med1* mutant was tested for complementation by cloned wild-type genes identified by mutants in which meiotic recombination is reduced or eliminated (ENGEBRECHT and ROEDER 1989; HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990, 1991; KLAPHOLZ, WADDELL and ESPOSITO 1985; ENGEBRECHT, HIRSCH and ROEDER 1990; MENEES and ROEDER 1989; BHARGAVA, ENGEBRECHT and ROEDER 1991; MALONE *et al.* 1991). The results indicate that *med1-1* is not an allele of *HOP1*, *MER1*, *RED1*, *MEK1*, *SPO11*, *MER2*, *MEI4*, *REC102*, *REC104* or *REC114* (data not shown).

***MED1* defines a new epistasis group:** There are three known epistasis groups among mutants displaying decreased levels of meiotic recombination. Mutations in one group, defined by mutants that do not undergo any meiotic recombination, are epistatic to the others (PETES, MALONE and SYMINGTON 1991). The reduced levels of meiotic prototrophs found in *med1* strains provides an assayable feature to conduct

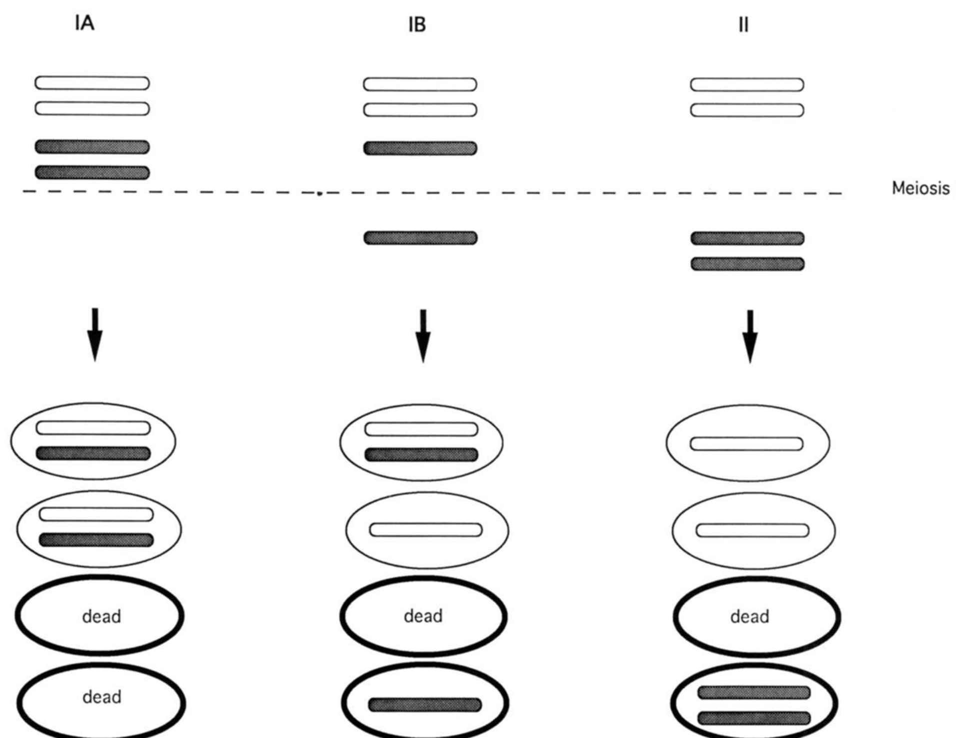


FIGURE 1.—Missegregation of chromosomes during meiosis. (IA) Both pairs of homologs segregate to the same pole at meiosis I. A normal equational segregation of the homologs at meiosis II results in two viable sister spores, which are disomic. (IB) One homolog and one chromatid from the homologous chromosome segregate to the same pole at meiosis I (*i.e.*, a three-to-one segregation). At meiosis II, the intact homolog segregates equationally and the univalent segregates randomly to form a single disomic spore whose sister is viable. (II) A normal meiosis I followed by a meiosis II in which two sister chromatids fail to segregate from each other. This results in a single disomic spore whose sister is inviable.

epistasis tests with other meiotic mutants that also display partial recombination defects. *RED1*, *HOP1* and *MEK1* belong to one epistasis group (ROCKMILL and ROEDER 1990, 1991) and *MER1* defines another (ENGEBRECHT and ROEDER 1989). To determine whether *med1* is a member of the *RED1/HOP1/MEK1* group, a *mek1 med1 spo13* strain and a congenic *mek1 spo13* strain were constructed and assayed for meiotic prototroph formation. The *mek1 med1* mutant displays a greater reduction in meiotic prototroph production than either single mutant (Table 5). *mer1 med1 spo13* and congenic *mer1 spo13* strains were also analyzed (Table 5). Again, the *med1 mer1 spo13* mutants displayed a greater reduction in meiotic prototroph production than either single mutant. Thus, *MED1* belongs to neither the *RED1/HOP1/MEK1* nor the *MER1* epistasis group.

The relationship between recombination and nondisjunction: To examine the relationship between recombination and chromosome segregation in the *med1* homozygote, spores that have inherited two copies of the same chromosome (n + 1) due to nondisjunction were isolated and then analyzed to determine whether nondisjoined chromosomes had sus-

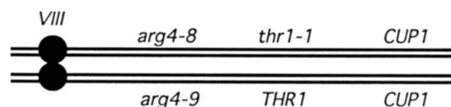
TABLE 3

Tetrad types from *med1* diploids

Strain (chromosome)	IA	IB	II	IB or II	IA or IB	Total tetrads
BR2837 (*VIII*)	0	4	1	0		330
BR2837 (*III*)	4	0	1	2		330
BR2877 (*III*)	4	0	5	0	4	320

For BR2837, IA indicates a tetrad containing two viable sister spores, both of which are disomic. IB indicates tetrads in which two or three spores are viable; one spore is disomic and its sister is viable. II indicates a three-spore viable tetrad containing one disomic spore whose sister is not viable. IB or II indicates a two-spore viable tetrad in which one spore is disomic and a nonsister spore is viable. In addition, there were eight tetrads in which a single spore was viable and disomic for chromosome *VIII* and 10 tetrads in which the single viable spore was disomic for chromosome *III*.

For BR2877, IA indicates tetrads containing two viable disomic spores, in which both spores are Ura^+ Trp^+. II indicates a tetrad containing one or two viable spores, one of which is disomic and either Ura^+ Trp^- or Ura^- Trp^+. IA or IB indicates a tetrad in which one spore is viable and a Ura^+ Trp^+ disome.



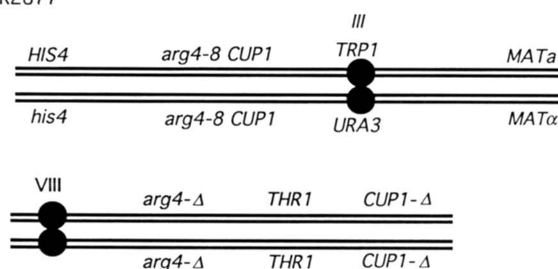


FIGURE 2.—Configuration of markers in strains BR2837 and BR2877.

TABLE 4

Crossing-over in *spo13* strains

	HIS4-LEU2 (cM)	*LEU2-MAT* (cM)	*ADE6-CYH3* (cM)	*CYH3-TRP5* (cM)	*TRP5-MET13* (cM)
MED1/*med1*	20	33	28	14	39
med1/*med1*	13	15	16	7	32
Fold decrease	1.5×	2.2×	1.8×	2×	1.2×

Data from five congenic Med$^+$ strains, BR2804-BR2808, and from two congenic Med$^-$ strains, BR2809-BR2810, were pooled. From wild type, 224 two-spore viable dyads were scored; 99 two-spore viable dyads were scored from mutant. Recombination in the chromosome *III* intervals was scored as described by ENGEBRECHT and ROEDER (1989). Recombination in the *CYH3-ADE6* and *CYH3-TRP5* intervals was determined as for the *CYH10-LYS2* interval (ENGEBRECHT and ROEDER 1989). Dyads that displayed $1^+:1^-$ segregation for *TRP5* but $2^+:0^-$ segregation for *MET13*, or vice versa, were assumed to result from crossovers in the *TRP5-MET13* interval. Dyads that could be accounted for either by a single crossover or by more than one crossover were assumed to be due to single exchanges.

TABLE 5

Meiotic gene conversion

Strain	Relevant genotype	Prototroph frequency (×10^{-5}) *leu2-27*/*leu2-3 112*	*thr1-1*/*thr1-4*	*trp1-1*/*trp1-289*	*his4-260*/*his4-519*
BR2777-2779 BR2783-2785	*MED1*/*med1*	45	25	11	670
BR2774-2776 BR2780-2782	*med1*/*med1*	7.6 (5.9×)	6.8 (3.7×)	5.6 (2×)	470 (1.4×)
BR2801	*MED1*/*med1* *mek1*/*mek1*	1.8 (25×)	1.2 (21×)		83 (8.1×)
BR2800	*med1*/*med1* *mek1*/*mek1*	0.39 (115×)	0.53 (47×)		32 (21×)
BR2822	*MED1*/*med1* *mer1*/*mer1*	0.53 (85×)	0.47 (53×)		
BR2821	*med1*/*med1* *mer1*/*mer1*	0.11 (409×)	0.19 (132×)		

The *MED1/med1* values are the averages from BR2777-BR2779 and/or BR2783-BR2785. The ***med1/med1*** values are the averages from BR2774-BR2776 and/or BR2780-BR2782. The first three Med$^+$ and Med$^-$ strains are heteroallelic for *LEU2, THR1* and *HIS4* and the last three diploids in each set are heteroallelic for *LEU2, THR1* and *TRP1*. All of these strains have one parent in common and the other parents are congenic due to multiple backcrosses to a wild-type strain. The ***mek1*** and ***mer1*** strains are isogenic with BR2777 or BR2774, but homozygous for the corresponding disruption allele. The meiotic prototroph frequencies were calculated by subtracting the premeiotic value from the meiotic value and averaging the corrected frequencies from three to six independent cultures of each genotype. The numbers in parentheses indicate the fold decrease from the corresponding wild-type value.

tained a crossover. The frequency of crossing over (map distance) was then compared with that derived from spores containing a single copy of the same chromosome, due to proper chromosome segregation (ROCKMILL and FOGEL 1988; ROCKMILL and ROEDER 1990; ENGEBRECHT, HIRSCH and ROEDER 1990).

Spores disomic for chromosome *VIII* were isolated from BR2837 on the basis of copper-resistance, and recombination in the *ARG4-THR1* interval on chromosome *VIII* was measured (MATERIALS AND METHODS). Disomic spores homoallelic at *ARG4* displayed the same frequency of recombination as monosomes (Table 6). In contrast, recombination was markedly reduced in disomic spores heteroallelic at *ARG4* (Table 6). Most of the heteroallelic disomes presumably resulted from nondisjunction at meiosis I, since *ARG4* is centromere-linked (15 cM). The homoallelic disomes resulted either from meiosis II nondisjunction or from crossing over in the *CENVIII-ARG4* interval followed by meiosis I nondisjunction. The fraction of disomic spores that were homoallelic at *ARG4* corresponded approximately to the frequency expected to result from meiosis II nondisjunction based on tetrad analysis (Table 3).

To further explore the relationship between recombination and nondisjunction at the two meiotic divisions, disomic spores from BR2877 (Figure 2) were recovered and analyzed. In this strain, disomes generated at each division are distinguishable because the centromeres are marked. Recombination was scored

TABLE 6

Recombination in *med1* monosomic and disomic spores

A. BR2837	Monosome *VIII* *ARG4-THR1*	Disome *VIII* *ARG4-THR1* *arg4-8/arg4-9*	Disome *VIII* *ARG4-THR1* *arg4-8/arg4-8, arg4-9/arg4-9*
	19.7 cM (47/239)	1.6 cM (7/426)	20.5 cM (25.5/124)
B. BR2877	**Monosome *III* *CENIII-MAT***	**Disome *III* *CENIII-MAT* MI**	**Disome *III* *CENIII-MAT* MII**
	15.1 cM (55/365)	5.7 cM (37/646)	18.0 cM (31/172)

A. Monosomes and some disomes for chromosome *VIII* were isolated by screening random spores for copper-resistance (method I, MATERIALS AND METHODS). Most of the disomes were isolated by selection on copper-containing medium (method II). Recombinants were scored as described in MATERIALS AND METHODS.

B. *CENIII-MAT* map distances for spores monosomic for chromosome *III* were calculated from a random spore analysis of BR2877. Spores disomic for chromosome *III* were isolated by method II, and map distances were calculated as described in MATERIALS AND METHODS. Numbers in parentheses are the number of recombinant chromatids over the total number of chromatids.

in the *CENIII-MAT* interval. Consistent with the studies of chromosome *VIII* (Table 6), recombination in the disomes resulting from meiosis I nondisjunction was less frequent than recombination in monosomes or in disomes resulting from meiosis II nondisjunction. These results suggest that recombination does not influence the probability of nondisjunction at meiosis II, but that reduced recombination is associated with missegregation at meiosis I.

***med1* mutants form apparently wild-type SC:** Most previously identified meiotic mutants display some defect in the formation or abundance of SC. Analysis of the SC was accomplished by spreading sporulating cells, staining with silver nitrate and viewing the stained chromosomes in the electron microscope (DRESSER and GIROUX 1988). Spreads from congenic *MED1/med1* (BR2777) and *med1/med1* (BR2774) diploids are displayed in Figures 3A, 3B and 3C. Mutant and wild-type spreads were indistinguishable; both contained full length SCs and a single, darkly staining nucleolus. In most SCs, two lateral elements, representing the proteinaceous backbones of the individual chromosomes, were observed as parallel structures. The frequency of pachytene nuclei (containing fully synapsed chromosomes) was similar in mutant and wild type at a number of different time points (data not shown).

The SC and the nucleolus are the predominant structures evident with silver staining. To examine chromosome condensation, spreads were stained with the DNA-binding dye, DAPI, and observed in the light microscope. Spreads of wild-type and *med1/med1* sporulating diploids are displayed in Figures 4A and 4B. The chromosomes from *med1/med1* and wild-type

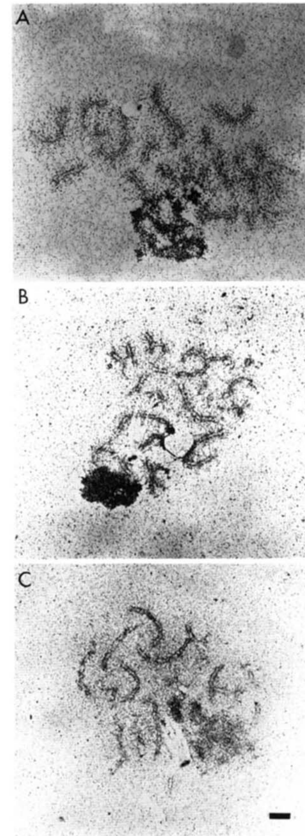


FIGURE 3.—Electron micrographs of meiotic nuclear spreads. (A) Wild-type (BR2777) and (B and C) *med1/med1* (BR2774) nuclei during pachytene, showing fully synapsed homologous chromosomes. Bar = 1 μm.

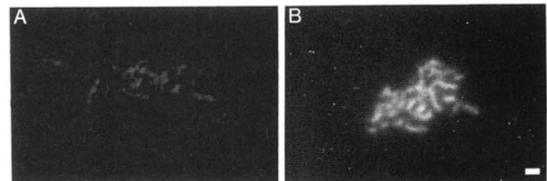


FIGURE 4.—DAPI-stained meiotic nuclei. (A) Wild type (BR2777) and (B) *med1/med1* (BR2774) nuclei displaying fully condensed "sausage-like" chromosomes. Bar = 1 μm.

strains are indistinguishable. At the stage of maximum chromatin condensation, individual chromosomes appear as "sausages" in both wild-type and mutant spreads.

DISCUSSION

The *med1* mutant displays a novel meiotic phenotype. Diploids homozygous for the *med1* mutation form cytologically normal SC and recombine at nearly wild-type levels. The poor spore viability (20%) observed in the mutant cannot be explained entirely by the reduction in crossing over. Even chromosomes that have recombined nondisjoin in *med1* homozygous strains.

About 5% of the spores from a *med1* homozygote are disomic for chromosome *III* or *VIII*. Assuming a

frequency of nondisjunction of 5% per chromosome per meiosis, we would expect 42% of the tetrads to have four viable spores. We observe 6% four-spore viable tetrads. However, a nondisjunction frequency of 5% per chromosome is probably a gross underestimate of the actual frequency since it is based on the analysis of viable spores. Aneuploidy is presumably more frequent among inviable meiotic products. Thus, we believe that the observed spore lethality can be attributed entirely to chromosome missegregation leading to aneuploidy.

med1 homozygotes missegregate chromosomes at both meiosis I and meiosis II. Nondisjunction at meiosis I occurs in two ways (Figure 1): either both homologs segregate to the same pole (IA), or one homolog and one chromatid segregate to one pole and the remaining chromatid segregates to the other pole (IB). Although the data in Table 3 show differential segregation for chromosomes *III* and *VIII*, we believe that this is due to the small number of informative tetrads. If the data from the two strains and two chromosomes are pooled, we calculate that approximately two-thirds of the disomes derived from meiosis I nondisjunction are due to type IA missegregations. Missegregation at meiosis II, like type IB missegregation at meiosis I, involves the precocious separation of sister chromatids. In the *med1* mutant, meiosis I nondisjunction is about three- to four-times as frequent as nondisjunction at meiosis II (Table 6).

Recombination in the meiosis II-generated disomes occurs at similar levels as in monosomes, suggesting that recombination does not influence segregation at meiosis II. Disomes derived from missegregation at meiosis I display less than a third as much recombination as monosomes. Type IA segregation is the type of segregation expected for chromosomes in which no crossovers took place (and consequently no chiasmata were formed). If type IA disomes are nonrecombinant, then the overall frequency of recombination in the meiosis I (IA and IB) disomes would be reduced, as observed. Thus, we suggest that there are two defects in the *med1* mutant. As a result of the reduction in reciprocal crossing over, some chromosomes fail to recombine and therefore undergo type IA nondisjunction. In addition, sister chromatids separate precociously leading to missegregation of types IB and II. We propose that the *med1* mutation leads to an alteration in chromosome (or chromatin) structure and that the observed defects in sister chromatid cohesion and recombination are secondary consequences of this alteration.

Even though missegregation occurs at both meiotic divisions in *med1* homozygotes, the primary defect might be restricted to meiosis I. If sister chromatids separate precociously during meiosis I, they presumably will segregate independently. Half of the time, the two sisters will move to the same pole at meiosis I (*i.e.*, segregate correctly); these chromatids have a 50% chance of moving to the same pole at meiosis II (*i.e.*, segregating incorrectly), resulting in type II missegregation. Thus, all of the observed meiosis II missegregation events may result from sister chromatid separation prior to the first division. A defect confined to meiosis I is consistent with the spore viability of the *med1 spo13* diploid, since *spo13* diploids skip meiosis I (KLAPHOLZ and ESPOSITO 1980). An alternative hypothesis is that precocious sister chromatid separation occurs at both meiotic divisions in *SPO13* strains and in the meiosis II-like division in *spo13* diploids. In this case, the effect of precocious separation of sister chromatids on viability in *med1 spo13* diploids may be counteracted by the decrease in recombination. Mutations that reduce crossing over generally improve spore viability in *spo13* diploids (MALONE and ESPOSITO 1981; ROCKMILL and ROEDER 1988, 1991), but the *med1* mutation has no such effect.

A number of mutants of Drosophila, maize, Sordaria and yeast have been postulated to be defective in meiotic sister chromatid cohesion (MASON 1976; GETHMANN 1984; GOLDSTEIN 1980; KERREBROCK *et al.* 1992; MIYAZAKI and ORR-WEAVER 1992; MAGUIRE 1978; MOREAU, ZICKLER and LEBLON 1985; ROCKMILL and FOGEL 1988; FLATTERS and DAWSON 1993). Of the Drosophila mutants, the defect in the *ord* mutant is proposed to occur earlier than the *meiS-332* and *mei-G87* defects because there is a higher proportion of meiosis I missegregations relative to meiosis II missegregations (MASON 1976; GETHMANN 1984; GOLDSTEIN 1980; KERREBROCK *et al.* 1992; MIYAZAKI and ORR-WEAVER 1992). The relative frequency of type IB segregations to type II segregations in the *med1* strains, approximately one to one, makes this mutant phenotypically very similar to *ord* and is consistent with an early loss of sister chromatid cohesion.

MAGUIRE (1990) has proposed that the SC is responsible for installing or reinforcing meiotic sister chromatid cohesion. Sister chromatids often separate precociously in certain translocation strains or trisomics, in which centromeric regions are not synapsed. In some organisms, structures that appear to be SC remnants, have been observed between sister chromatids along their length at metaphase I (MOENS and CHURCH 1979). Two mutants proposed to be defective in sister chromatid cohesion, *spo76* in *Sordaria* and *desynaptic* in maize (MOREAU, ZICKLER and LEBLON 1985; MAGUIRE, PARADES and REISS 1992), display aberrant SCs. The *spo76* mutant displays little tripartite SC and the axial elements are often split as if each chromatid forms an axial element (MOREAU, ZICKLER and LEBLON 1985). The *desynaptic* mutant forms SC with an expanded distance between the

lateral elements (MAGUIRE, PARADES and REISS 1992). Although *med1* mutants form apparently normal SCs, it is possible that there is a subtle alteration of this structure that is not readily detected in our spread preparations. An alteration is the SC would be consistent with the observed decrease in genetic recombination.

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