Meiosis in Asynaptic Yeast

Beth Rockmill and G. Shirleen Roeder

Department of Biology, Yale University, New Haven, Connecticut 06511-8112

Manuscript received February 21, 1990
Accepted for publication July 24, 1990

ABSTRACT

The *Saccharomyces cerevisiae red1* mutant fails to assemble synaptonemal complex during meiotic prophase. This mutant displays locus-specific reductions in interchromosomal gene conversion and a moderate reduction in crossing over. The occurrence of a significant amount of meiotically induced recombination in the *red1* mutant indicates that the synaptonemal complex is not absolutely required for meiotic exchange. The *RED1* gene product is required for intrachromosomal recombination in some assays but not others. Chromosomes that have undergone reciprocal exchange nevertheless nondisjoin in *red1* mutants, indicating that crossovers are not sufficient for disjunction. Epistasis studies reveal that *HOP1* is epistatic to *RED1*, and that *RED1* acts in an independent pathway from *MER1*. A model for the function of the *RED1* gene product in chromosome synapsis is discussed.

MEIOSIS is distinguished from the mitotic cell division in several respects. During prophase I of meiosis, homologous chromosomes pair with each other and undergo high levels of genetic recombination. Two rounds of chromosome segregation ensue in contrast to the single equational division that occurs in mitosis. The meiosis I reductional segregation, in which homologous chromosomes disjoin, precedes an equational segregation, in which sister chromatids separate and segregate. Together, the two rounds of chromosomal disjunction generate four haploid nuclei.

Meiotic recombination is correlated with the presence of the cytologically observable structure called the synaptonemal complex (SC) (reviewed in VON WETTSTEIN, RASMUSSEN and HOLM 1984). During SC assembly, an axial element is formed along each pair of sister chromatids. The SC is formed when the axial elements pair with each other (and become lateral elements) and a central core is laid down between them. The SC is conventionally thought to be responsible for the alignment of homologous chromosomes as a precondition for recombination. Mutants that abolish or severely restrict meiotic recombination are often found to lack SC (*rad50*, ALANI, PADMORE and KLECKNER 1990; *spo11*, DRESSER, GIROUX and MOSES 1986; *mer1*, ENGEBRECHT and ROEDER 1990; *c(3)G*, SMITH and KING 1968). In several organisms, recombination is restricted to limited segments of chromosomes, and SC is found only in these segments (see VON WETTSTEIN, RASMUSSEN and HOLM 1984 for review). Some organisms that lack recombination, such as male *Drosophila melanogaster*, also lack SC (COOPER 1950).

An alternative to the view that SC assembly is a precondition for recombination is that the initiation of recombination may occur prior to SC formation, as suggested by MAGUIRE (1988) and CARPENTER (1987). Consistent with this hypothesis, some organisms that undergo normal levels of meiotic recombination do not have SC (OLSON and ZIMMERMANN 1978; EGEL-MITANI, OLSON and EGEL 1982). In addition, the *hop1* and *mer1* mutants of yeast fail to assemble SC, but do undergo significant levels of meiotic recombination (approximately 10% of wild type) (HOLLINGSWORTH and BYERS 1989; ENGEBRECHT and ROEDER 1989, 1990). Correlative cytological and genetic evidence suggests an enzymatic role in recombination for recombination nodules which are located at intervals along the SC (CARPENTER 1975). Nodules have also been observed during the early stages of pairing at association points between axial elements (ANDERSON and STACK 1988; ALBINI and JONES 1987). If one role of recombination nodules is to initiate synapsis by promoting gene conversion events (CARPENTER 1987), it could explain why recombination-defective mutants fail to synapse.

We are interested in defining gene products required for meiosis I chromosome segregation in an attempt to better understand the relationships between chromosome pairing, genetic recombination and meiosis I disjunction. A mutant at the *RED1* locus was recovered in a screen for meiotic lethal mutants (ROCKMILL and ROEDER 1988). *red1* mutants fail to segregate their chromosomes properly at the reductional division of meiosis. Unfortunately, the DNA sequence of the *RED1* gene provided no insight into the function of the *RED1* gene product (THOMPSON and ROEDER 1989). In the analysis of *red1* null mutants presented here, we have detected phenotypes indicating that the *RED1* gene product is required for chromosome synapsis.

TABLE 1

Yeast strains

BR1373-6D	*MAT***a** *leu2-27 his4-280 arg4-8 thr1-1 ura3-1 trp1-1 cyh10^R ade2-1*
BR1919-8B	*MATα leu2-3,112 his4-260 thr1-4 ura3-1 trp1-289 ade2-1*
BR2487	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 red1::LEU2* *MAT***a** *leu2-27 HIS4 ura3-1 trp1-289 spo13::URA3 arg4-8 THR1 CYH10 ade2-1 red1::LEU2*
BR2495	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 ARG4 thr1-4 cyh10^R ade2-1* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 arg4-8 thr1-1 CYH10 ade2-1*
BR2482	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R LYS2 ade2-1 red1::LEU2* *MAT***a** *leu2-27 HIS4 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 lys2-98 ade2-1 red1::LEU2*
BR2483	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R LYS2 ade2-1* *MAT***a** *leu2-27 HIS4 ura3-1 trp1-289 spo13::URA arg4-8 thr1-1 CYH10 lys2-98 ade2-1*
BR2500	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 red1::ADE2*
BR2533	*MATα CDC2 LEU2 his4-260 ura3-1 trp1-1 arg4-9 THR1 cyh10^R lys2-98 ade2-1 red1::ADE2* *MAT***a** *cdc2 leu2-27 HIS4 ura3-1 trp1-1 arg4-8 thr1-1 CYH10 LYS2 ade2-1 red1::ADE2*
BR2541	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 mer1::LEU2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 mer1::LEU2*
BR2542	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 mer1:LEU2 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 mer1::LEU2 red1::ADE2*
BR2543	*MATα CENIII::HIS3 leu2-3, 112 his4-260 ura3-52 his3-11, 15 lys1-1 TRP1 spo13::URA3 arg4-17 ade1 ADE2* *MAT***a** *CENIII::HIS3 leu2-2 his4-712 ura3-52 his3-11, 15 lys1-1 trp1-1 spo13::URA3 arg4-17 ADE1 ade2-1*
BR2544	*MATα CENIII::HIS3 leu2-3, 112 his4-260 ura3-52 his3-11, 15 lys1-1 TRP1 spo13::URA3 arg4-17 ade1 ADE2 red1::LEU2* *MAT***a** *CENIII::HIS3 leu2-2 his4-712 ura3-52 his3-11, 15 lys1-1 trp1-1 spo13::URA3 arg4-17 ADE1 ade2-1 red1::LEU2*
BR2545	*MATα ΔCENIII::HIS3 leu2-3, 112 bik1:CENIII-211 his4-260 ura3-52 his3-11, 15 lys1-1 TRP1* *MAT***a** *ΔCENIII::HIS3 leu2-2 bik1::CENIII-211 his4-712 ura3-52 his3-11, 15 lys1-1 trp1-1* *spo13::URA3 arg4-17 ade1 ADE2* *spo13::URA3 arg4-17 ADE1 ade2-1-BIK1-ADE2*
BR2546	*MATα ΔCENIII::HIS3 leu2-3, 112 bik1::CENIII-21his4-260 ura3-52 his3-11, 15 lys1-1 TRP1* *MAT***a** *ΔCENIII::HIS leu2-2 bik1:CENIII-211 his4-712 ura3-52 his3-11, 15 lys1-1 trp1-1* *spo13::URA3 arg4-17 ade1 ADE2 red1::LEU2* *spo13::URA3 arg4-17 ADE1 ade2-1-BIK1-ADE2 red1::LEU2*
J114	*MATα cdc10-2 LEU2-URA3-CYH2-HIS4* *MAT***a** *CDC10 leu2 his4* *trp1 ura3 can1 spo13-1 ade2-1 sap3 lys2-99 cyh2^R*
BR2547	*MATα cdc10-2 LEU2-URA3-CYH2-HIS4* *MAT***a** *CDC10 leu2 his4* *trp1 ura3 can1 spo13-1 ade1 sap3 lys2-99 cyh2^R red1::ADE2*
BR2554	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 hop1::TRP1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 hop1::TRP1 RED1*
BR2555	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 hop1::TRP1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 hop1::TRP1 red1::ADE2*
BR2558	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 red1::ADE2* *pB8 B8(RED1, URA3)*
BR2559	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 red1::ADE2* *pB93(red1-1, URA3)*
BR2560	*MATα leu2-3, 112 his4-260 ura3-1 trp1-1 spo13::URA3 ARG4 thr1-4 cyh10^R ade2-1 red1::ADE2* *MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 spo13::URA3 arg4-8 thr1-1 CYH10 ade2-1 red1::ADE2* *pB94(red1-2, URA3)*
BR2561	*MATα leu2-3, 112 HIS4-ura3-Stu-his4-260 ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1* *MAT***a** *leu2-27 his4-280* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1*
BR2562	*MATα leu2-3, 112 HIS4-ura3-Stu-his4-260 ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2* *MAT***a** *leu2-27 his4-280* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade21 red1::ADE2*
BR2570	*MATα leu2-27 his4-912-URA3-his4-260-3′Δ ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2* *MAT***a** *LEU2 his4-Δ401* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 RED1*
BR2571	*MATα leu2-27 his4-912-URA3-his4-260-3′Δ ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2* *MAT***a** *LEU2 his4-Δ401* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2*
BR2572	*MATα leu2-27 his4-290-URA3-his4-260 ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2* *MAT***a** *LEU2 his4-Δ401* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 RED1*
BR2573	*MATα leu2-27 his4-290-URA3-his4-260 ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2* *MAT***a** *LEU2 his4-Δ401* *ura3-1 trp1-1 spo13::ura3-1 arg4-8 thr1-1 ade2-1 red1::ADE2*

MATERIALS AND METHODS

Media, yeast strains and plasmids: Media and genetic manipulations are described by SHERMAN, FINK and HICKS (1986). Yeast strains are listed in Table 1. Isogenic diploids of the BR2495 series were constructed by transforming the two haploids, BR1373-6D and BR1919-8B, with various plasmids and then mating the appropriate transformants. Transformations with the following five plasmids resulted in gene disruptions (ROTHSTEIN 1983) and these transformants were checked by Southern blot analysis. In pB72, a segment of the *RED1* gene has been deleted and replaced by the *LEU2* gene (ROCKMILL and ROEDER 1988). pB84 contains an insertion of the 3.6-kb *ADE2* gene with *Bam*HI linkers into the *Bgl*II site of *RED1* in pR849 (THOMPSON and ROEDER 1989). pMEΔ162 contains a deletion-disruption of the *MER1* gene marked with *LEU2* (ENGEBRECHT and ROEDER 1989). pNH32-2 contains a *HOP1* gene disrupted by *TRP1* (HOLLINGSWORTH and BYERS 1989). pSpo13(Δ16) carries a deletion-disruption of *SPO13* marked with *URA3* (WANG *et al.* 1987). *spo13::ura3-1* alleles were made by plating haploid strains bearing the *spo13::URA3* and *ura3-1* mutations on medium containing 5-fluoro-orotic acid (FOA) (BOEKE, LACROUTE and FINK 1984). The *URA3* gene was converted to a *ura3-1* allele by ectopic recombination. These strains cannot generate Ura$^+$ recombinants, indicating that the *URA3* genes are homoallelic.

The following plasmids were targeted for integrative transformation. pAZ2a was used to integrate a *ura3* allele into chromosome *III*. pAZ2a (made by E. LAMBIE, unpublished results) consists of a 1.5-kbp *Sal*I fragment containing the 5′-end of the *HIS4* gene inserted at the *Sal*I site of pBR322 and a 1.1-kbp *Hin*dIII fragment containing the *URA3* gene inserted at the *Hin*dIII site. The *URA3* gene was rendered nonfunctional by inserting a *Xho*I linker at the *Stu*I site. pV100 and pR37 were used to make *HIS4* duplications. pV100 contains a 2.8-kbp *Eco*RI-*Sal*I fragment carrying the 5′ end of the *HIS4* gene marked with the *his4-260* mutation inserted between the *Eco*RI and *Sal*I sites of YIp5. pR37 contains a 13.2-kbp fragment extending from the *Eco*RI site upstream of *HIS4* to the *Bam*HI site downstream of the gene; *HIS4* carries the *his4-260* mutation.

Strains BR2543 and BR2544 are derivatives of J101-T30$^+$ and J95-T30P (*CENIII* at its normal position) and BR2545 and BR2546 are derived from J101-T55A$^+$ and J95-T55B (in which *CENIII* has been moved to the *HIS4* locus) (LAMBIE and ROEDER 1988). Since *BIK1* is disrupted by the insertion of *CENIII-211* in the transpocentric strains, this mutation was complemented by inserting *BIK1* at *ADE2*. First, an *ade2-Bgl* mutation was made by transformation of J95-T55B with pR493. pR493 contains the 3.6-kbp *Eco*RI-*Bam*HI (linker) fragment containing *ade2* (with the *Bgl*II site filled in) inserted between the *Eco*RI and *Bam*HI sites of YIp5. This strain was then transformed with pR866 targeted for integration at *ADE2*; pR866 contains the 3.1-kbp *Eco*RI fragment of *BIK1* in the *Eco*RI site of pBR325 and the 3.6-kbp *Bgl*II fragment of *ADE2* fragment flanked by *Bam*HI linkers in the *Bam*HI site. In addition, all four strains were made *spo13* by transformation with pSpo13(Δ16).

Cloning the *red1-1* and *red1-2* alleles: pB8 [YCp50 with the original 10-kbp insert containing *RED1* (ROCKMILL and ROEDER 1988)] was cut with *Xba*I and religated, resulting in a complete deletion of *RED1* coding sequences. The resulting plasmid, pB99, was cut with *Xba*I and transformed into the original *red1-1* isolate and a diploid containing another UV-induced allele, *red1-2* (J. ENGEBRECHT, unpublished results). Plasmids were recovered from yeast by running yeast DNA minipreps on agarose gels and isolating the appropriately sized DNA from the gel with Gene Clean (Bio 101) and transforming *Escherichia coli*. Plasmids with restriction patterns similar to the original pB8 were named pB93 (*red1-1*) and pB94 (*red1-2*). When these plasmids were transformed into homozygous *red1::ADE2* diploid strains, they failed to complement the *red1* defect.

Growth of cells for sporulation: Strains not containing episomal (CEN) plasmids were grown in rich medium (YEPD supplemented with adenine) 24–36 hr before inoculation into 2% potassium acetate. Strains containing YCp50-derived plasmids were grown overnight on solid SC-ura medium. The following morning, a large inoculum was placed in rich medium for approximately 10 hr, and then diluted (1:10) into 2% potassium acetate. Plasmid loss frequency was determined when cells were harvested and was generally 2–5%.

Cytology: Cells used for cytological studies were spheroplasted prior to sporulation (ALANI, PADMORE and KLECKNER 1990) as follows. Two ml of cells from early stationary phase in YPAD (ROCKMILL and ROEDER 1988) were pelleted, resuspended in 2 ml 200 mM Tris (pH 7.6) and 80 mM dithiothreitol (made fresh) and incubated at room temperature for 5 min. The cells were then pelleted and resuspended in 2 ml 50 mM Tris (pH 7.6), 0.5 M potassium chloride and 0.05–0.25 mg/ml Zymolyase 100T (icn). After 10 min rocking at room temperature, the cells were centrifuged at low speed. The pellet was gently resuspended in 1 ml osmotically stabilizing sporulation medium (0.5 M potassium chloride, 2% potassium acetate); the cell suspension was then poured into 9 ml of the same medium in a 250-ml Erlenmeyer flask and gently shaken (150 rpm) at 30° for 17–23 hr. Cells were then harvested and prepared for examination in the electron microscope using the general protocol from DRESSER and GIROUX (1988) as modified by ENGEBRECHT and ROEDER (1990).

RESULTS

***red1* mutant alleles:** To examine the phenotypes of *red1* null mutants, two alleles were used. The *red1::LEU2* allele is a deletion-disruption allele missing approximately two-thirds of the amino-terminal coding region plus the 5′ upstream sequences (THOMPSON and ROEDER 1989; ROCKMILL and ROEDER 1988) and is assumed to be a null allele. The *red1::ADE2* allele is an insertion of the *ADE2* gene at the *Bgl*II site in the center of the *RED1* coding region. The *red1::LEU2* and the *red1::ADE2* alleles behave similarly with respect to spore viability and levels of meiotic gene conversion (data not shown); we therefore assume that both alleles represent null mutations. To compare the

Footnote to Table 1:

BR2482 and BR2483 are isogenic. BR2487, BR2495, BR2500, BR2541, BR2542, BR2554, BR2555, BR2558, BR2559 and BR2560 are isogenic. His$^+$ and Thr$^+$ recombinants were selected in BR2487. BR2543, BR2544, BR2545 and BR2546 are isogenic. J114 and BR2547 are isogenic to KarC2 (HOLLINGSWORTH and BYERS 1989). BR2561 and BR2562 are isogenic. BR2570 and BR2571 are isogenic and have pV100 integrated on one copy of chromosome *III*. The isogenic strains BR2572 and BR2573 were derived by genetic crosses from a haploid *his4-290* strain transformed with pR37. Plasmids are described in MATERIALS AND METHODS.

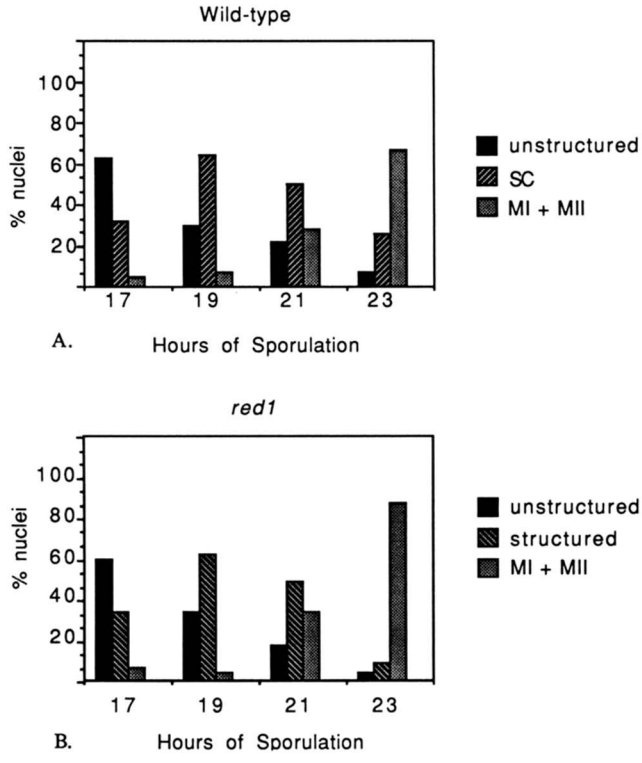

FIGURE 1.—Time course of meiosis. Cells from BR2495 (A) or BR2500 (B) were sporulated and nuclei spread as described in MATERIALS AND METHODS. Cells were harvested at 2-hr intervals from 17 to 23 hr into meiosis. Nuclei scored as "unstructured" contain duplicated but unseparated spindle pole bodies (SPBs) and diffuse chromatin. Nuclei scored as having SC contain paired lateral elements (see Figure 2A) and were only found in wild type. "Structured" nuclei are those in the *red1* mutant (B), containing thin stained structures (see Figure 2, B, C and D). MI and MII represent nuclei during the two chromosomal divisions; these nuclei have diffuse chromatin and either a single pair of separated SPBs or two pairs of SPBs.

two UV-induced alleles to the null mutants, isogenic strains carrying the original allele, *red1-1* (ROCKMILL and ROEDER 1988), and a newly isolated allele, *red1-2* (J. ENGEBRECHT, unpublished results), were constructed. The *red1-1* and *red1-2* mutations were cloned by gap repair on a CEN plasmid (MATERIALS AND METHODS) and then transformed into a *red1::ADE2* diploid.

***red1* mutants do not make SC:** Recent advances in spreading yeast meiotic nuclei for electron microscopy provide a relatively simple method for visualization of SC (DRESSER and GIROUX 1988). Meiotic cells from wild type and *red1* mutants were prepared and spread as described in MATERIALS AND METHODS. Of the wild-type cells harvested at 19–21 hr after introduction into sporulation medium, 50–65% were in pachytene (the time of complete synapsis) (Figure 1). A typical pachytene spread from wild type is shown in Figure 2A. The SC is evident along the axes of the bivalents, where the silver stain detects the proteinaceous components of the two lateral elements. Other nuclear structures such as the nucleolus and the duplicated but unseparated spindle pole bodies can also be seen. In contrast, normal SC was not observed among more than 1000 spreads of *red1* nuclei. Examination of mutant nuclei spread at times when most wild-type cells are in pachytene, revealed areas of relatively intense staining (Figure 2, B–D). Note that the micrographs of the *red1* nuclei are shown at a lower magnification than the wild-type nucleus because the chromatin in *red1* spreads is more diffuse. Mutant and wild type exhibit similar kinetics of sporulation if nuclei containing darkly stained regions in the mutant are considered to be at an equivalent stage to the nuclei containing SC in wild type (Figure 1).

***red1* mutants retain substantial amounts of meiotic recombination:** The induction of meiotic recombination can be measured by crossover frequencies (map distances) between marked loci and by prototroph frequencies at heteroallelic loci. The effect of *red1* mutations on recombination can be assayed in the viable spores of a *spo13 red1* double mutant (ROCKMILL and ROEDER 1988). Diploids homozygous for a *spo13* mutation skip meiosis I chromosome segregation and undergo a single, predominantly equational, division (KLAPHOLZ and ESPOSITO 1980). Consequently, the *spo13* mutant restores viability to *red1* and some other meiotic mutants with defects in meiosis I (*spo11*, MALONE and ESPOSITO 1981; *rad50*, MALONE 1983; *mer1*, ENGEBRECHT and ROEDER 1989; *hop1*, HOLLINGSWORTH and BYERS 1989; *mei4*, MENEES and ROEDER 1989).

Meiotic intragenic recombination (gene conversion) was measured in isogenic *spo13* strains carrying four pairs of heteroalleles. Diploid strains carrying one of three *RED1* alleles (*red1::ADE2*, *red1-1* or *red1-2*) were compared for prototroph frequencies. The four pairs of heteroalleles studied display a wide range of meiotic induction levels. All *red1* alleles have similar effects on intragenic recombination although the *red1-2* allele appears to have a slightly stronger effect. Meiotic prototroph frequencies at three loci are severely reduced in *red1* strains relative to wild type (Table 2). Recombination at *TRP1*, however, occurs at wild-type levels in the *red1* mutants.

Meiotic intergenic recombination was measured in *spo13* strains by dyad analysis (Table 3). A diploid strain homozygous for the *red1::LEU2* allele was compared to an isogenic wild type for recombination between the *HIS4* and *MAT* loci on chromosome *III* and between *CYH10* and *LYS2* on chromosome *II*. The map distances in both intervals are reduced approximately four-fold in the *red1::LEU2* mutant. The frequencies of both aberrant and reductional segregations in the *red1* dyads are decreased with respect to wild type, consistent with the behavior of other meiotic recombination mutants (*e.g. spo11*, KLAPHOLZ, WADDELL and ESPOSITO 1985; *rad50*, MALONE 1983;

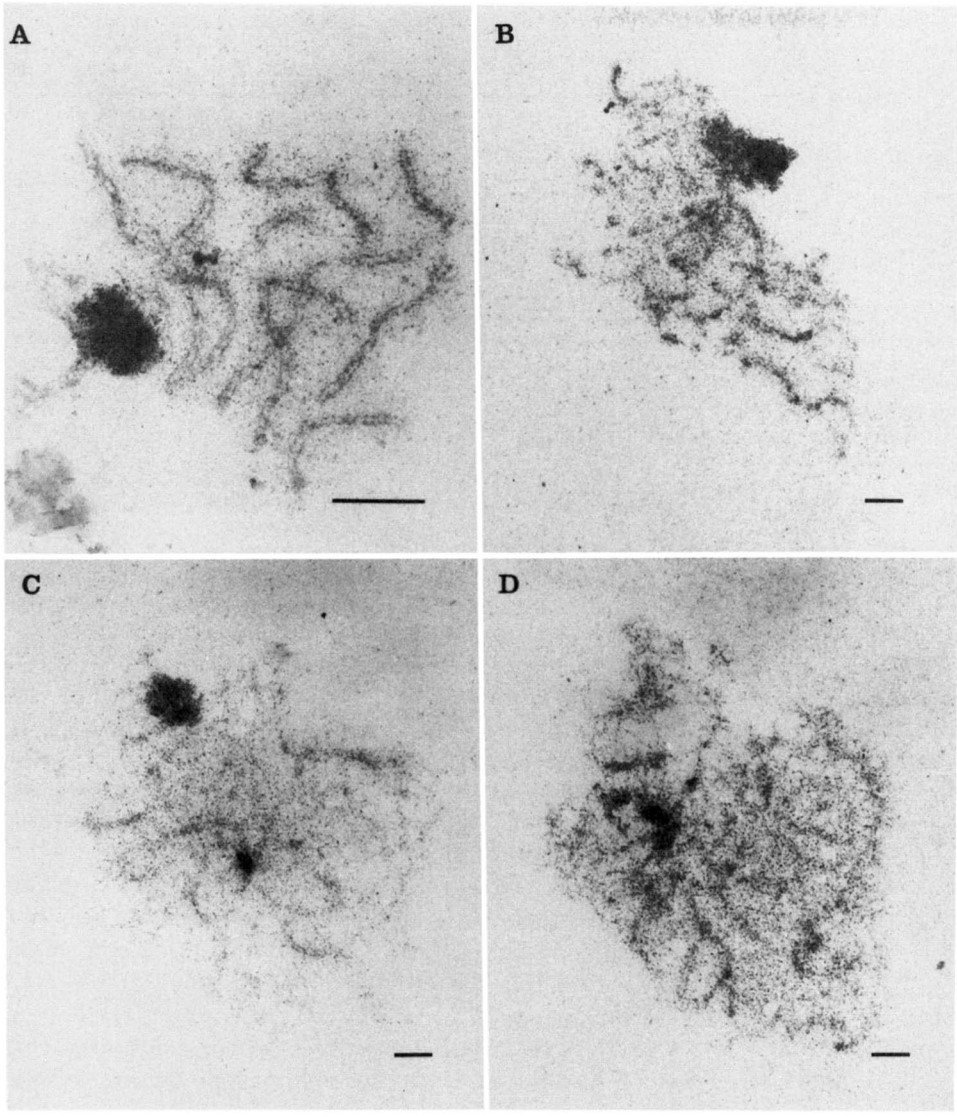

FIGURE 2.—Electron micrographs of meiotic nuclei. Nuclei were prepared and spread as described in MATERIALS AND METHODS. A is a micrograph of a pachytene nucleus from wild type (BR2495). Pairs of lateral elements can be seen along most chromosomes. B, C and D are micrographs of nuclei from a *red1* mutant (BR2500) shown with chromatin at the most condensed stage. Large darkly staining areas are the nucleoli; duplicated but unseparated spindle pole bodies can be seen in A, B and D (*e.g.*, see B upper left corner). Bar = 1 μm.

TABLE 2

Meiotic intragenic recombination in *spo13* strains

Strain	Relevant genotype	*leu2-27* / *leu2-3,112* (× 10^{-6})	-Fold decrease	*his4-280* / *his4-260* (× 10^{-6})	-Fold decrease	*thr1-1* / *thr1-4* (× 10^{-6})	-Fold decrease	*trp1-1* / *trp1-289* (× 10^{-6})	-Fold decrease
BR2558	*RED1*	700	1×	6200	1×	510	1×	100	1×
BR2500	*red1::ADE2*	13	54×	540	11×	14	36×	86	1.2×
BR2559	*red1-1*	13	54×	370	17×	16	32×	99	1×
BR2560	*red1-2*	4.2	167×	260	24×	11	46×	47	2.1×

The rate of meiotic prototrophy was determined by subtracting the mitotic (premeiotic) frequency from the meiotic frequency for each experiment and averaging the meiotic values. At least four experiments were done for each strain. The -fold decreases were determined by dividing the mean meiotic frequency of the wild type by the frequency observed in the mutant. The median mitotic frequencies for the four strains were approximately 9×10^{-8}, 4.7×10^{-5}, 4.0×10^{-7} and 5.6×10^{-7} for *LEU2*, *HIS4*, *THR1* and *TRP1*, respectively.

mer1, ENGEBRECHT and ROEDER 1989; and *mei4*, MENEES and ROEDER 1989; *hop1*, HOLLINGSWORTH and BYERS 1989). It is difficult to reach any firm conclusions regarding the effect of a *red1* mutation on crossing over since only two intervals were examined and the studies of gene conversion indicate that the effect of a *red1* mutation can vary from one region to another. Since crossing over measures exchange throughout a relatively large interval, the map distances may be averages representing some regions that are profoundly affected and others that are not.

Recombination in transpocentric strains: Recombination at the *TRP1* locus, unlike recombination at the other loci tested, appears to be *RED1*-independent. One feature which might account for the unexpected behavior of *TRP1* is its proximity to the cen-

TABLE 3

Meiotic intergenic recombination in *spo13* strains

Strain	Genotype	Percent spo. viab.	#2-spo. viab.	% Aber. seg. III	Red. seg. III	*HIS4-MAT* T:NPD / total	*HIS4-MAT* (cM)	% Aber. seg. II	Red. seg. II	*CYH10-LYS2* T:NPD / total	*CYH10-LYS2* (cM)
BR2483	*RED1*	77	122	5.7	6	38:1 / 115	38.3	5.7	2	21:1 / 115	23.5
BR2482	*red1::LEU2*	80	144	2.1	1	12:0 / 141	8.5	0.7	0	9:0 / 143	6.3

Dyads that displayed aberrant segregation (Aber. seg.) for the chromosome in question were eliminated from the map distance calculation (*i.e.*, dyads containing either one mater and one nonmater, or one cycloheximide-resistant spore and one cycloheximide-sensitive spore capable of producing resistant recombinants). The number of dyads with reductionally segregating chromosomes (Red. seg.) is shown for chromosomes *III* and *II* as red. seg. III and II, respectively. Map distances were calculated as described by ENGEBRECHT and ROEDER (1989). Chromosome *III* recombinant dyads had the following phenotypes: His^+ nonmater:His^- nonmater or His^+ **a**:His^+ α. Four-strand double crossovers (NPD) segregated His^+ α:His^- **a** when the two crossovers occurred on opposite sides of the centromere; this represents the majority of the 4-strand double crossovers. Chromosome *II* recombinant dyads had the following phenotypes after equational segregation: Cyh^{pap} Lys^+: Cyh^{pap} Lys^- (^{pap} = resistant papillae). Recombinant dyads resulting from a reductional segregation had the following phenotypes: Cyh^R Lys^+: Cyh^S Lys^+. Four-strand double exchanges (Cyh^R Lys^-: Cyh^S Lys^+) can be detected only if chromosomes segregate reductionally and therefore the *CYH10-LYS2* map distances are minimal estimates. Map distances were calculated as follows: map distance = [single crossovers +6(NPD)]/total × 100. This equation accounts for the fact that half of the recombination events that are followed by equational chromosome segregation escape detection. spo. = spore; aber. = aberrant; red. = reductional; pap = papillae; T = tetratype; NPD = nonparental ditype.

TABLE 4

Intragenic recombination in normal and transpocentric strains

Strain	Relevant markers	Meiotic His^+ ($\times 10^{-6}$)	-Fold decrease
BR2543	Red^+ normal CEN	12,000	1×
BR2544	*red1::ADE2* normal CEN	2,800	4.3×
BR2545	Red^+ transpocentric	910	1×
BR2546	*red1::ADE2* transpocentric	130	7×

In strains BR2545 and BR2546, a 211-bp fragment containing *CENIII* is moved proximal to the *HIS4* gene on chromosome *III*. Prototroph frequency was measured between the *HIS4* alleles, *his4-260* and *his4-712*. Premeiotic values for histidine prototrophy were subtracted from the meiotic values.

tromere; *TRP1* maps 0.5 cM from *CENIV*. The effect of *RED1* on recombination in another centromere-adjacent interval was examined in strains in which the centromere of chromosome *III* had been deleted from its normal location and transposed to the *HIS4* locus, 50 kbp away. Previous studies of recombination in normal strains and strains homozygous for a transposed centromere (transpocentric) demonstrated that meiotic gene conversion at *HIS4* is decreased in transpocentric strains, indicating that recombination at *HIS4* is subject to centromeric repression of meiotic recombination (LAMBIE and ROEDER 1988).

The *red1::LEU2* mutation was introduced into normal and transpocentric strains and the meiotic frequencies of His^+ recombinants were then determined (Table 4). The *red1::LEU2* mutation reduces recombination at *HIS4* in both sets of strains indicating that the *RED1* gene product is required for recombination at *HIS4* whether or not the gene is centromere-adjacent. Thus, it seems unlikely that the *RED1*-independence of recombination at *TRP1* is a consequence of its centromere-proximity.

Intrachromosomal recombination: The effect of a *red1* mutation on recombination between repeated sequences present on the same chromosome was examined using three assays. The assay developed by HOLLINGSWORTH and BYERS (1989) measures intrachromosomal "popout" recombination. This assay utilizes a *spo13* haploid strain disomic for chromosome *III* to measure the frequency of crossing over between direct repeats located on a single copy of chromosome *III* between *LEU2* and *HIS4*. Recombination between the repeats can result in excision of a *CYH2* gene, leaving the cell cycloheximide-resistant due to the recessive *cyh2*^{R} mutation on chromosome VIII (Figure 3A). A *red1::ADE2* derivative displays wild-type levels of meiotic intrachromosomal recombination in this assay (Table 5A).

Two assays were used to measure intrachromosomal gene conversion at *HIS4*. Both assays involve a duplication of chromosome *III* sequences with the *URA3* gene and pBR322 sequences inserted between the repeats (see Figure 3, B and C). Assay I (Figure 3B) measures recombination between a truncated gene carrying the *his4-912* allele and a complete gene carrying the *his4-260* mutation. The meiotic frequency of His^+ recombinants is 12-fold lower than wild type in an isogenic *red1::ADE2* derivative (Table 5B).

Assay II also involves *HIS4* but the region of homology is larger (13.2 kbp) and prototrophs result from recombination between the *HIS4* genes, *his4-260* and *his4-290* (Figure 3C). Both the wild-type and *red1::ADE2* strains display similar meiotic frequencies (Table 5B). In wild type, reciprocal recombination measured by the frequency of Ura^- spores (FOA^r) is not significantly induced meiotically in either assay I or II (data not shown).

Ectopic recombination: Ectopic recombination refers to genetic exchange between homologous se-

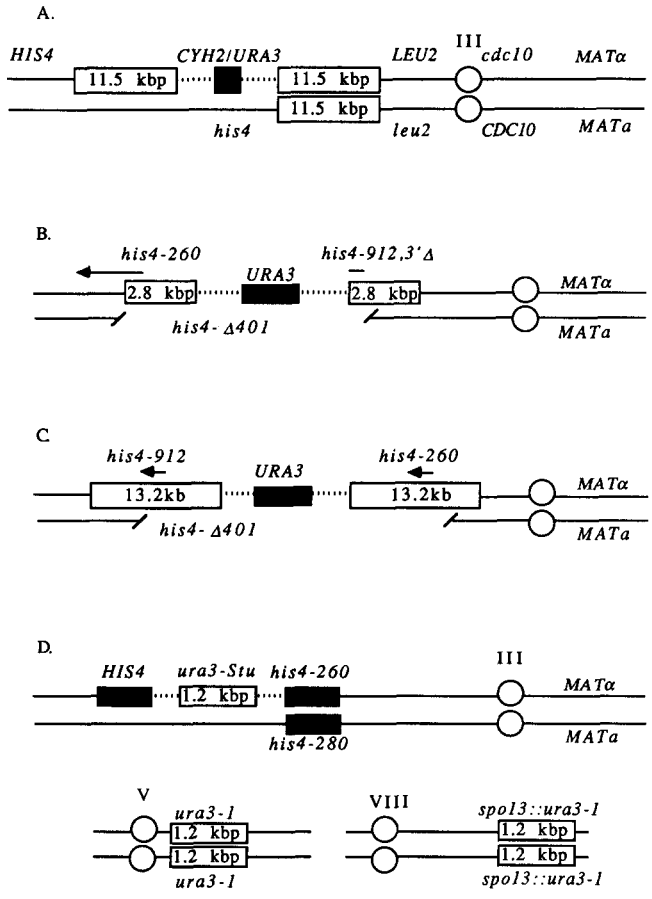

FIGURE 3.—Assays used to measure intrachromosomal and ectopic recombination. A, Intrachromosomal assay. pNH18, containing the *URA3* and *CYH2* genes and 11.5 kbp of chromosome *III* DNA, is integrated into chromosome III. Recombinants are cycloheximide-resistant (see HOLLINGSWORTH and BYERS 1989). B, Intrachromosomal gene conversion assay I. pV100 is integrated on chromosome *III*. His$^+$ recombinants result from intragenic recombination between a truncated gene carrying the *his4-912* allele and a complete gene carrying the *his4-260* allele. The *his4-Δ401* allele is a deletion that covers all *HIS4* alleles used in this assay and assay II. C, Intrachromosomal gene conversion assay II. pR37 is integrated on chromosome III. His$^+$ recombinants result from recombination between the *HIS4* heteroalleles. D, Ectopic assay. pAZ2a is integrated on chromosome III. Recombination between the *ura3-Stu* gene on chromosome *III* and a *ura3-1* allele on either chromosome *V* or *VIII* may result in a Ura$^+$ recombinant. Circles represent centromeres. Open boxes represent homologous sequences capable of recombination in the various assays. Black boxes represent yeast sequences inserted near duplicated regions. Arrows indicate the *HIS4* coding region. Broken lines indicate vector sequences.

quences on nonhomologous chromosomes. Ectopic recombination can be meiotically induced 10–100-fold (LICHTEN, BORTS and HABER 1987; JINKS-ROBERTSON and PETES 1985). The role of the *RED1* gene product in ectopic recombination was examined in diploid strains carrying a *URA3* gene marked with the *ura3-Stu* mutation at the *HIS4* locus on one copy of chromosome *III*. The strain is homozygous for the *ura3-1* mutation on chromosome *V* and homozygous for a *SPO13* gene disrupted with *ura3-1* on chromosome *VIII* (see MATERIALS AND METHODS) (Figure 3D).

TABLE 5

Intrachromosomal recombination in *red1* mutants

A. Popouts

Strain	Relevant genotype	Mitotic Cyh^r ($\times 10^{-4}$)[b]	Meiotic Cyh^r ($\times 10^{-4}$)[b]	Corrected[a] ($\times 10^{-4}$)	-Fold decrease
J114	*RED1*	3.5	54	216	1×
BR2547	*red1::ADE2*	4.6	320	320	0.7×

B. Gene Conversion

Strain	Relevant genotype	Mitotic His$^+$ ($\times 10^{-5}$)[b]	Meiotic His$^+$ ($\times 10^{-5}$)[b]	-Fold decrease
Assay I				
BR2570	*RED1* / *red1::ADE2*	5.9	310	1×
BR2571	*red1::ADE2* / *red1::ADE2*	3.0	25	12×
Assay II				
BR2572	*RED1* / *red1::ADE2*	60	1100	1×
BR2573	*red1::ADE2* / *red1::ADE2*	51	1000	1.1×

[a] Corrected meiotic frequency is the meiotic frequency multiplied by the reciprocal of the equational segregation frequency. This calculation is made because recombination events are detected only in those dyads in which chromosome *III* segregates equationally (see HOLLINGSWORTH and BYERS 1989). J114 displays 25% equational segregation for chromosome *III* (ENGEBRECHT and ROEDER 1989), as does kar-C2-4, the disome from which J114 was derived (HOLLINGSWORTH and BYERS 1989). BR2547 displays 100% equational segregation for chromosome *III* (46/46 dyads).

[b] Recombination frequencies were calculated as in Table 2.

The frequency of Ura$^+$ spores from the wild-type strain is seven-fold higher than the frequency from the isogenic *red1* derivative (Table 6), demonstrating that the *RED1* gene product is required for ectopic recombination in this assay.

Crossovers do not ensure disjunction in *red1* mutants: Genetic exchange is thought to ensure proper chromosome disjunction at the reductional division through the formation of chiasmata. Although *red1* mutants have reduced levels of crossing over, a significant amount of exchange still occurs. To determine whether the crossovers that occur in a *red1* mutant ensure disjunction, the rare viable spores derived from a *red1 SPO13* meiosis were examined for exchange between chromosomes that nondisjoined and the map distance obtained was compared to that derived from spores monosomic for the chromosome in question. If exchange ensures disjunction, then chromosomes that have undergone nondisjunction should be nonrecombinant and the apparent map distance between two genes should be greatly reduced among disomic spores. Conversely, if exchange has no effect on disjunction, then the map distance in the disomic spore population should be similar to that calculated from spores carrying a single copy of the chromosome.

The map distance between *ARG4* and *THR1* on

TABLE 6

Ectopic recombination

Strain	Relevant genotype	Mitotic Ura$^+$ ($\times 10^{-6}$)	Meiotic Ura$^+$ ($\times 10^{-6}$)	-Fold decrease
BR2561	*RED1* / *RED1*	6.6	135	1×
BR2562	*red1::ADE2* / *red1::ADE2*	5.9	20	6.8×

chromosome *VIII* was measured in spores from a *red1 SPO13* diploid. The diploid starting strain was heteroallelic for two complementing *ARG4* alleles, *arg4-8* and *arg4-9*. *arg4-8* is a temperature-sensitive allele and confers arginine prototrophy below 30°. Strains homozygous for the *arg4-9* allele are auxotrophic at all temperatures. Diploids or disomes carrying both the *arg4-8* and *arg4-9* alleles are prototrophic up to 35° (ROCKMILL and FOGEL 1988). Because the nondisjunction occurring in the *red1* mutant takes place at the reductional division (ROCKMILL and ROEDER 1988) and because *ARG4* is centromere-linked (15 cM), most spores disomic for chromosome *VIII* are Arg$^+$. In addition, the dosage-sensitive copper resistance gene, *CUP1^S*, is homozygous in BR2533, permitting an independent measure of disomy (ROCKMILL and FOGEL 1988). To measure exchange in disomes, threonine auxotrophy was scored among the copper-resistant Arg$^+$ disomic spores (*THR1* maps distal to *ARG4*). Arg$^+$ Thr$^-$ spores are indicative of a crossover between *ARG4* and *THR1* and represent one-quarter of the recombinant chromatids (Figure 4).

In this experiment, approximately equal numbers of monosomic or disomic spores (for chromosome *VIII*) were recovered from the *red1* mutant. The map distance for *ARG4-THR1* among disomic spores was 9.8 cM. This value is slightly more than half the map distance derived from monosomic segregants of the same diploid (17.8 cM) (Table 7). Thus, crossovers do not always ensure disjunction in a *red1* background; however, a crossover increases the probability that a chromosome pair will disjoin properly by a factor of 2.

Epistasis between *red1* and other meiotic mutants: *mer1* mutants are defective in meiotic recombination and form axial elements but not SC (ENGEBRECHT and ROEDER 1989, 1990). The *hop1* mutant is defective in meiotic recombination and fails to form SC (HOLLINGSWORTH and BYERS 1989). In an attempt to place these genes in epistasis groups, *red1 mer1* and *red1 hop1* mutants were constructed and analyzed for allelic recombination at three loci (Table 8). Although both *red1* and *mer1* single mutants are meiotically induced for the production of prototrophs at all three loci, the double mutant shows no increase in frequency above the mitotic background level. Thus, the *red1* and *mer1* mutations act synergistically, suggesting that the *RED1* and *MER1* gene products act in different pathways. The *hop1 red1* strain exhibited a level of meiotic recombination similar to *hop1*. Therefore, *HOP1* and *RED1* act in the same pathway and *HOP1* is epistatic to *RED1*.

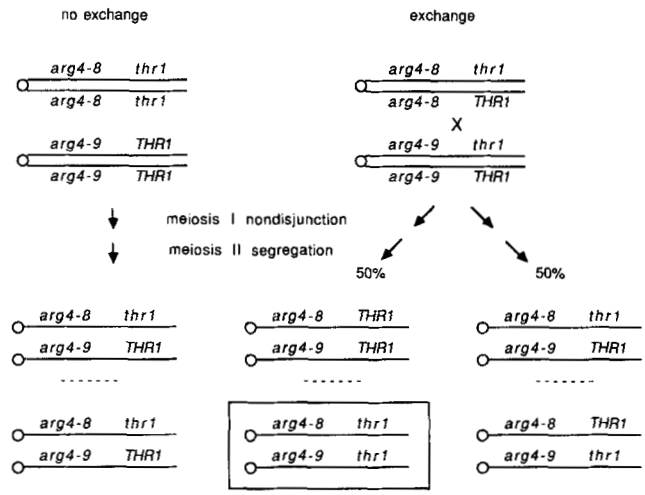

FIGURE 4.—Generation of disomic spore products after nondisjunction at meiosis I. Meiotic products represent disomic spores. Half the meioses in which recombination between *ARG4* and *THR1* is followed by meiosis I nondisjunction and normal meiosis II disjunction, result in one Arg$^+$ Thr$^-$ spore (shown in the box).

DISCUSSION

A *red1* mutation causes alterations in meiotic recombination and SC assembly: The *red1* mutant was previously described as a meiotic lethal mutant defective in meiosis I chromosome segregation but recombination-proficient (ROCKMILL and ROEDER 1988). Surprisingly, a cytological examination of *red1* mutants revealed a failure of wild-type SC production. *red1* mutants undergo meiosis with similar kinetics to the wild type but, at the time when wild-type nuclei display SC, *red1* nuclei appear relatively unstructured. Although there are no obvious axial elements or SC there are regions of intense staining which may be fragments of axial elements or even tripartite SC. This result provoked a further examination of meiotic recombination in *red1* mutants.

As found previously for the *red1-1* mutant, allelic recombination at *TRP1* in the *red1* null mutant occurs at wild-type levels. In contrast, allelic recombination measured at three other loci is reduced 11- to 53-fold in a *red1* null mutant. Thus, a *red1* mutant affects recombination differentially at different loci. In measurements of meiotic map distance, a *red1* null mutation reduced recombination fourfold in two intervals (*HIS4-MAT* and *CYH10-LYS2*). In contrast, the *red1-1* mutant reduced the map distance in the *HIS4-MAT* interval by only 20% (ROCKMILL and ROEDER 1988), suggesting that the *red1-1* allele is leaky. Due to the

TABLE 7

Crossing over and meiotic chromosome segregation

Random meiotic products	Total meiotic products analyzed	Number exchanges	Frequency crossing over (cM)
Haploid spores	191	34	17.8
Disomic spores	449	22	9.8

Random spores were isolated from BR2533 by plating on cycloheximide-containing medium. (BR2533 is heterozygous for *cyh10*^{R} and *CYH10* is less than 0.5 cM from *CENII*.) Haploid spores were distinguished by copper sensitivity and either arginine auxotrophy (*arg4-9*) or temperature sensitivity (*arg4-8*). Disomic spores were distinguished by copper resistance and arginine prototrophy at 35° (see text). The *ARG4-THR1* map distance in haploids was calculated by multiplying the frequency of recombinants by 100. The map distance among random disomic spores was calculated by multiplying the frequency of Thr$^-$ spores by 200.

region-specific effects of the *red1* mutation and the leakiness of the *red1-1* allele, the *red1* mutant was previously incorrectly diagnosed as recombination-proficient.

The *red1* mutant is indistinguishable from wild type in three different assays of meiotic recombination: (1) allelic recombination at *TRP1*, (2) intrachromosomal crossing over, and (3) one assay for intrachromosomal gene conversion (assay II). The observation that *red1* mutants undergo a significant amount of meiotically-induced recombination implies that meiotic exchange does not absolutely require cytologically detectable SC.

Based on the phenotype of the *red1* mutant, it is possible to speculate about the function of the *RED1* gene product. The *RED1* gene product is apparently required for the assembly of axial elements (and therefore SC assembly); the *RED1* protein could be a structural component of axial elements or it might play catalytic role in the assembly of these structures. Alternatively, the *RED1* gene product may play a direct role in recombination which may lead to a defect in synapsis as suggested by CARPENTER (1987) and MAGUIRE (1988). However, the observation that *red1* mutants are proficient in several different recombination assays suggests that the recombination machinery is intact. The defect in axial element formation may be the cause of the observed alterations in synapsis and recombination. For example, changes in chromosome architecture in the *red1* mutant may reduce the accessibility of DNA to recombination enzymes and/or to the apparatus that promotes chromosome synapsis. Finally, it is possible that the *RED1* gene product controls the expression and/or activity of a number of genes or gene products involved in meiotic recombination and chromosome synapsis.

Genetic exchange is not sufficient for chromosome disjunction: Disomic spores from *red1 SPO13* meioses were analyzed and the nondisjoined chromosomes were found to display a significant amount of recombination. Thus, the exchange events in a *red1* mutant are not sufficient to ensure proper chromosome disjunction at the reductional division. Similar results were obtained with the *mer1* mutant (ENGEBRECHT, HIRSCH and ROEDER 1990). One possible explanation is that the *red1* mutant is defective in some function required to convert a crossover into a functional chiasma. The results of ENGEBRECHT, HIRSCH and ROEDER (1990) suggest that the missing function may be associated with the SC.

Chiasmata are the cytological manifestations of genetic exchange and correspond to chromatin bridges between nonsister chromatids. Chiasmata are thought to be essential for proper meiosis I chromosome segregation. Remnants of the SC or recombination nodules have been reported to be associated with chiasmata in several organisms (reviewed in VON WETTSTEIN, RASMUSSEN and HOLM 1984). Perhaps this SC-derived material keeps the bivalent intact until anaphase I, when homologous chromosomes dissociate.

Studies of other meiotic mutants provide additional support for the observation that crossovers are not sufficient for reductional segregation. For example, the *desynaptic* mutant of maize undergoes apparently normal levels of meiotic recombination (assayed cytologically) yet univalents are present at the metaphase I plate and homologues nondisjoin at anaphase I (MAGUIRE 1978). The existence of this mutant argues that more than just genetic exchange is required to establish functional chiasmata. The *ds* mutant appears to be defective in sister chromatid cohesion.

Diploid *spo13* mutants in yeast undergo predominantly equational chromosome segregation in meiosis even though they undergo normal levels of meiotic recombination (KLAPHOLZ and ESPOSITO 1980). Thus, a wild-type level of exchange in this mutant background does not force a reductional division. However, mutations that reduce or abolish the amount of meiotic exchange decrease nondisjunction and the frequency of reductional segregation in *spo13* strains, indicating that exchange does influence segregation in *spo13* mutants (*spo11*, KLAPHOLZ, WADDELL and ESPOSITO 1985; *mer1*, ENGEBRECHT and ROEDER 1989; *hop1*, HOLLINGSWORTH and BYERS 1989; *mei4*, MENEES and ROEDER 1989; *red1*, this paper).

The *RED1* gene product plays a role in recombination between nonallelic genes: Meiotic recombination can take place between duplicated sequences in nonallelic positions on the same chromosome (intrachromosomal recombination) (JACKSON and FINK 1985) as well as between sequences present on nonhomologous chromosomes (ectopic recombination) (JINKS-ROBERTSON and PETES 1985; LICHTEN, BORTS and HABER 1987). In the assay used in this

TABLE 8

Meiotic intragenic recombination in multiple mutants

Strain	Relevant genotype	*leu2-27* / *leu2-3,112* (× 10^{-6})	-Fold decrease	*his4-280* / *his4-260* (× 10^{-6})	-Fold decrease	*thr1-1* / *thr1-4* (× 10^{-6})	-Fold decrease	*trp1-1* / *trp1-289* (× 10^{-6})	-Fold decrease
BR2558	*RED1*	700	1×	6200	1×	510	1×	100	1×
BR2500	*red1::ADE2*	13	54×	540	11×	14	36×	86	1.2×
BR2541	*mer1::LEU2*			205	30×	19	27×	7.2	14×
BR2542	*red1::ADE2* *mer1::LEU2*			30	206×	0.1	5100×	0.7	143×
BR2554	*hop1::TRP1*	2.6	269×	90	69×	3.1	165×		
BR2555	*red1::ADE2* *hop1::TRP1*	2.8	250×	87	71×	2.8	182×		

The rates of meiotic prototrophy were determined by averaging the meiotic values from at least four experiments. The fold decreases were determined as in Table 2. Data from BR2558 and BR2500 are from Table 2. The values for BR2542 were at mitotic levels.

study, normal levels of ectopic recombination require the *RED1* gene product. Since *red1* mutants are defective in SC assembly, one interpretation of these results is that the SC mediates recombination between nonhomologous chromosomes. Consistent with this hypothesis, transient stretches of SC have been observed in species such as allohexaploid wheat, which displays homeologous pairing (pairing between related chromosomes) (RILEY and KEMPANNA 1963). In addition, nonhomologous chromosomes pair and form SC in haploid plants (VON WETTSTEIN, RASMUSSEN and HOLM 1984) and yeast (unpublished observation). Perhaps these SC-mediated associations reflect recombination between dispersed repeated sequences.

Three assays were used to measure meiotic intrachromosomal recombination. Two of the assays exhibited wild-type recombination frequencies in *red1* strains but, the third assay exhibited a requirement for the *RED1* gene product. The two *RED1*-independent assays (one measuring popouts and one measuring gene conversion) had relatively large duplications (11.5 kbp and 13.2 kbp, respectively), whereas the *RED1*-dependent gene conversion assay had only a 2.8-kbp duplication. These observations are consistent with the *RED1*-dependence found in the ectopic recombination assay, which measures recombination between 1.2-kbp homologous segments.

An interpretation of these results is that the *RED1* gene product is required for intrachromosomal recombination between short repeats, but not longer ones. Large repeats may have a high probability of finding each other due to an increased chance of random collision. In contrast, intrachromosomal (or ectopic) recombination between relatively small regions of homology may require a more facilitated pairing process, provided (directly or indirectly) by the *RED1* gene product. A possible role for the *RED1* gene product in promoting the pairing of homologous chromosomes is discussed below.

The *HOP1* and *RED1* gene products act in a different pathway from the *MER1* protein: In yeast, mutations at many loci affect meiotic exchange. Most of these mutations completely eliminate meiotic recombination (*spo11*, KLAPHOLZ, WADDELL and ESPOSITO 1985; *rad50*, GAME *et al.* 1980; *mei4*, MENEES and ROEDER 1989), but two (*hop1*, HOLLINGSWORTH and BYERS 1989; and *mer1*, ENGEBRECHT and ROEDER 1989) undergo some meiotically induced recombination. For the latter mutants, it is possible to examine their epistatic relationships with *red1*. The relationship of *red1* with *hop1* and *mer1* was established by comparing meiotic allelic recombination in the single and double mutants. Since the double mutant, *red1 hop1*, is similar in phenotype to the single *hop1* mutant, *HOP1* is epistatic to *RED1* (*i.e.*, acts before *RED1* in the same pathway). The *red1 mer1* mutant does not induce meiotic recombination, suggesting that these genes function in independent pathways. ENGEBRECHT and ROEDER (1989) found that *hop1 mer1* double mutants also do not induce meiotic allelic recombination. Thus, the pathway of meiotic recombination and chromosome synapsis diverges into parallel steps (one defined by *MER1*, the other by *HOP1* and *RED1*) which eventually converge to generate recombinant chromosomes with functional chiasmata.

A *red1* mutation has region-specific effects on allelic recombination: Measurements of intragenic recombination reveal that the effect of a *red1* mutation varies dramatically from one locus to another. A *red1* mutation reduces gene conversion at three loci examined, but recombination at *TRP1* is unaffected. The reason for this variation is probably not centromere proximity, since a *red1* mutation reduces recombination at *HIS4* even when *CENIII* is moved nearby.

TRP1 is located near *ARS1* where two mitotic scaffold-associated regions have been mapped (AMATI and GASSER 1988) and bent DNA has been found (SNYDER, BUCHMAN and DAVIS 1986). The scaffold can be thought of as the chromosome backbone and consists

of proteins associated at the bases of chromatin loops (AMATI and GASSER 1988). Bent DNA appears to be a basic feature of DNA sequences capable of specifically binding scaffolds (HOMBERGER 1989). It is possible that the mitotic scaffold is structurally related to the meiotic scaffold (*i.e.*, the axial elements of the SC) and that the same subset of sequences are found in the mitotic and meiotic scaffolds. If meiotic chromosome pairing is initiated by the alignment of axial elements, then the scaffold-associated sequences from nonsister chromatids would be in proximity during the initial stages of pairing, while the bulk of the chromatin would be outside the scaffold region and therefore unpaired. Since meiotic recombination occurs throughout the genome, there must be a mechanism to facilitate pairing of sequences situated far from the scaffold association sites. Perhaps it is this function which is mediated by the SC and altered in a *red1* mutant (this same function may promote intrachromosomal recombination between short repeats). In this model, the *red1* mutant would be proficient in recombination events between sequences at or near the scaffold, but other events would be reduced in proportion to their distance from the nearest scaffold binding site. Not consistent with this model is the observation that the *red1* mutant does affect recombination near a transposed centromere, even though centromeric sequences including *CENIII* are known to bind scaffolds (AMATI and GASSER 1988). It will be interesting to determine whether the 211-bp *CENIII* used in this study contains the identified scaffold binding site.

Summary: A *red1* mutation appears to be pleiotrophic, resulting in alterations in meiotic recombination, SC assembly and chiasma function. We suggest that the *RED1* gene product plays a role in SC assembly, possibly through the formation of axial cores, and that the observed defects in recombination and disjunction are a consequence of the failure of SC formation.

We would like to thank JAYA BHARGAVA, ERIC LAMBIE and KAREN VOEKEL-MEIMAN for providing strains and plasmids, and MIKE DRESSER, CRAIG GIROUX, JOANNE ENGEBRECHT, RUTH PADMORE and BARRY PIEKOS for valuable suggestions on the cytology. We thank JOANNE ENGEBRECHT, TOM MENEES and ADELAIDE CARPENTER for critical reading of the manuscript. This work was funded by American Cancer Society grant #MV-405.

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Communicating editor: N. KLECKNER