Coordination of the Initiation of Recombination and the Reductional Division in Meiosis in *Saccharomyces cerevisiae*

Kai Jiao, Steven A. Bullard, Laura Salem and Robert E. Malone

Department of Biological Sciences, University of Iowa, Iowa City, Iowa 52242

Manuscript received August 25, 1998
Accepted for publication February 9, 1999

ABSTRACT

Early exchange (EE) genes are required for the initiation of meiotic recombination in *Saccharomyces cerevisiae*. Cells with mutations in several EE genes undergo an earlier reductional division (MI), which suggests that the initiation of meiotic recombination is involved in determining proper timing of the division. The different effects of null mutations on the timing of reductional division allow EE genes to be assorted into three classes: mutations in *RAD50* or *REC102* that confer a very early reductional division; mutations in *REC104* or *REC114* that confer a division earlier than that of wild-type (WT) cells, but later than that of mutants of the first class; and mutations in *MEI4* that do not significantly alter the timing of MI. The very early mutations are epistatic to mutations in the other two classes. We propose a model that accounts for the epistatic relationships and the communication between recombination initiation and the first division. Data in this article indicate that double-strand breaks (DSBs) are not the signal for the normal delay of reductional division; these experiments also confirm that *MEI4* is required for the formation of meiotic DSBs. Finally, if a DSB is provided by the *HO* endonuclease, recombination can occur in the absence of *MEI4* and *REC104*.

THE initiation of recombination in meiosis is formally different from the initiation of recombination in prokaryotes or in phages. In *Escherichia coli* bacterial conjugation, for example, the entering male chromosome is "preinitiated"; it contains a broken end (*e.g.*, Ippen-Ihler and Minkley 1986). Likewise, in phage λ the cutting of the *cos* site provides a double-strand break (DSB) that can serve to initiate recombination (Thaler *et al.* 1987). In eukaryotes, however, broken DNA is carefully avoided during the mitotic cycle; indeed, broken DNA that is not repaired leads to a mitotic cell cycle arrest or lethality (Game 1993; Ivanov and Haber 1997; Kogoma 1997). Current evidence is consistent with the hypothesis that eukaryotic chromosomes entering meiosis are intact and strand breakage to initiate recombination must occur specifically in meiosis.

In the yeast *Saccharomyces cerevisiae*, ample data indicate that a key initiating event for meiotic recombination is the formation of many DNA DSBs in meiotic prophase I (*e.g.*, Cao *et al.* 1990; Sun *et al.* 1991; Goldway *et al.* 1993; Wu and Lichten 1994, 1995; Fan *et al.* 1995; Bullard *et al.* 1996). At least 11 genes, which have been called early exchange (EE) genes, are known to be required for the initiation of meiotic recombination (Mao-Draayer *et al.* 1996). Null mutations in most genes of the EE class completely abolish all forms of meiotic recombination; in these mutants, meiotic DSBs have not been detected [*SPO11* and *RAD50* (Cao *et al.* 1990); *XRS2* (Ivanov *et al.* 1992); *MRE11* (Johzuka and Ogawa 1995); *MER2* (Rockmill *et al.* 1995); *REC102*, *REC104*, and *REC114* (Bullard *et al.* 1996); *MRE2* (Nakagawa and Ogawa 1997)]. In our strain background, DSBs also were not detectable in cells containing mutations in two genes demonstrated to code for components of the synaptonemal complex, *HOP1* and *RED1* (Mao-Draayer *et al.* 1996).

Surprisingly, the failure in *S. cerevisiae* to initiate meiotic recombination does not result in a block in the progression of cells through meiosis. Even though the probability of forming a viable spore in the absence of recombination is very low, most cells continue through meiosis and proceed through both the first and second divisions (Petes *et al.* 1991). The overall percentage of cells forming mature ascospores and asci is reduced in diploids containing an EE mutation [*e.g.*, *mei4* (Menees *et al.* 1992); *rec102* (Bhargava *et al.* 1992; Cool and Malone 1992); *rec104* (Galbraith and Malone 1992); *rec114* (Pittman *et al.* 1993)]. The failure to stop meiosis when recombination does not initiate might suggest that there is no "communication" between the start of recombination and subsequent meiotic events.

We recently demonstrated in a set of isogenic strains that the initiation of meiotic recombination results in a normal delay of reductional division (Galbraith *et al.* 1997). We observed that cells containing mutations in several of the EE genes (*REC102*, *REC104*, and *REC114*) enter the first division approximately an hour earlier than wild-type (WT) cells. We found this intu-

Corresponding author: Robert E. Malone, Department of Biological Sciences, University of Iowa, Iowa City, IA 52242.
E-mail: robert-malone@uiowa.edu

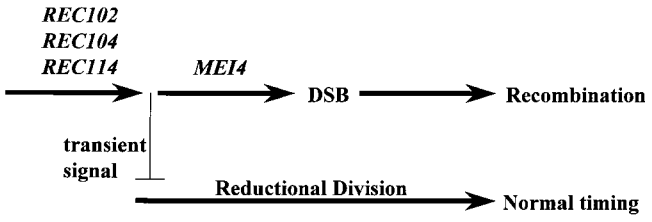

Figure 1.—Original model for the involvement of EE genes in the timing of reductional division (from Figure 6 of Galbraith *et al.* 1997). The initiation of recombination generates a transient signal that causes a delay of the first division. Some EE genes, including *REC102*, *REC104*, and *REC114* are required to form the transient signal. Mutations in these genes result in an earlier reductional division. The *MEI4* gene is an example of an EE gene that acts after the formation of the signal. *mei4* mutations do not significantly affect the timing of reductional division (Menees *et al.* 1992; Galbraith *et al.* 1997).

itively pleasing because it would allow time for recombination to occur before homologs separate from each other in the first division. We argued that the initiation of recombination creates a signal that results in the delay of the reductional division. Interestingly, mutations in one EE gene (*MEI4*) confer a different phenotype. As has been previously reported (Menees *et al.* 1992), *mei4* mutants entered the first division at a time indistinguishable from the time of WT cells (Galbraith *et al.* 1997). This effect on the timing of the reductional division was one of the first differences in meiotic phenotypes conferred by mutations in the EE genes. These data motivated us to propose a model for the order of action of these EE genes and the relationship of recombination to meiosis (Figure 1; Galbraith *et al.* 1997). As predicted by this model, mutations in the *REC104* gene were epistatic to mutations in the *MEI4* gene (Galbraith *et al.* 1997).

The experiments in this article address several questions raised by previous work that demonstrated a relationship between the initiation of meiotic recombination and the timing of the first meiotic division (Galbraith *et al.* 1997). All the EE genes examined in that article were meiosis-specific; that is, *MEI4*, *REC102*, *REC104*, and *REC114* are expressed only in meiosis (Cool and Malone 1992; Galbraith and Malone 1992; Menees *et al.* 1992; Pittman *et al.* 1993, respectively). We wondered whether mutations in the classic EE gene *RAD50*, which is required for mitotic recombination repair as well as for the initiation of meiotic recombination (Malone 1983; Alani *et al.* 1990; Cao *et al.* 1990; Johzuka and Ogawa 1995), also would result in an earlier reductional division. Although an elegant study of the timing of meiotic events has been published for the special allele *rad50S* (Alani *et al.* 1990), we could not find published data about the timing of the first division in a null *rad50* mutation.

The data in our initial work also suggested that *rec102* cells might enter the first division *even* earlier than *rec104* cells (Galbraith *et al.* 1997). In this article we ask whether that is true, and if there are in fact two classes of EE mutants that are "early" and "earlier" with respect to the timing of the first division. The previous results indicated that *rec104* mutations were epistatic to *mei4* mutations, consistent with *REC104* acting before *MEI4*. We ask whether other mutations are epistatic to *mei4*, as predicted by the model. Finally, the model shown in Figure 1 proposes that the creation of DSBs is not the signal that results in the delay of the first division. We test that in two ways. First, we ask whether DSBs occur in the *mei4* mutant. If breaks occur, then DSBs would remain a candidate for the signal between recombination and the first division. Second, we ask whether a specific DSB induced during meiotic prophase I in an EE mutant with an early reductional division can restore normal timing. The data in this article allow us to keep the general model for communication between the initiation of recombination and the first meiotic division but cause us to revise it to account for the new observations (see discussion).

MATERIALS AND METHODS

Plasmids: To obtain the *rec102-Δ2::LYS2* allele, a 2.7-kb *Eco*RI-*Kpn*I fragment from pCM208 (Cool and Malone 1992) containing *REC102* was cloned into pRS426 (Christianson *et al.* 1992) to generate pJK8. The 1.2-kb *Bsp*EI-*Bbu*I fragment of pJK8 containing *REC102* was then removed and replaced with the 4.8-kb *Eco*RI-*Pst*I fragment containing the *LYS2* gene from the YDp-K plasmid (Berben *et al.* 1991) to obtain pJK27. The deletion starts at bp −605 and ends at bp +609 of *REC102*.

pL32 and pJH10 were gifts from J. E. Haber (Brandeis University). pL32 contains the *pSPO13-HO* fusion (Malkova *et al.* 1996). To obtain a selectable marker for yeast transformation, the primers G418a (5′-CCCCGGTACCCAGCTGAAGC TTCGTACGC-3′) and G418b (5′-CCCCGGTACCGCATAGG CCACTAGTGGATTTG-3′), both of which contained a *Kpn*I restriction site at their ends, were used to amplify by PCR the P_{TEF}-*kan*^{r}-T_{TEF} fragment from pUG6 (Guldener *et al.* 1996). All oligonucleotide primers used in this study were synthesized at Integrated DNA Technologies (Coralville, IA). The amplified fragment was directly cloned into the unique *Kpn*I site at the 5′ end of the remaining *LYS2* coding region in pL32 to obtain pRM283, which contains the *lys2Δ::kan*^{r}*::pSPO13-HO* construct. pJH10 contains the *MATα-inc* allele, which is a single base pair substitution C:G → T:A at the Yα/Z border that inhibits the *HO* endonuclease digestion (Weiffenbach *et al.* 1983). To acquire a selectable marker for integrating the *MATα-inc* allele into our strain, a 6.4-kb *Eco*RI-*Eco*RI fragment of pJH10 containing *MATα-inc* was first cloned into pRS306 (Sikorski and Hieter 1989) in which the unique *Bam*HI site is disrupted (pRM280). Primers TRP5a (5′-GGAAAGAACTG GAT*c*CTCTAGACC) and TRP5b (5′-GTAACAATTGGATC*c* TATACGGTG-3′) were used to amplify by PCR a fragment containing the *TRP5* gene (−276 to +2556) from yeast genomic DNA; the fragment was cloned downstream of *MATα-inc* to generate pRM285. (*c* refers to a single base substitution to generate a *Bam*HI site.)

Strains: Genotypes of yeast strains are listed in Table 1. All strains for kinetic studies are isogenic and are the derivatives of the homothallic diploid K65-3D. All Rec$^-$ mutations (with the exception of *rad50S*) are null mutations that delete all or

TABLE 1

Yeast strains

Strain	Genotype	Reference
K65-3D	*HO MAT***a** *lys2-1 tyr1-1 his7-2 can1^r ura3-13 ade5 met13-d trp 5-2 leu1-12 ade2* *HO MAT*α *lys2-1 tyr1-1 his7-2 can1^r ura3-13 ade5 met13-d trp 5-2 leu1-12 ade2*	Galbraith *et al.* (1997)
K65-104-4A	Isogenic to K65-3D except *rec104-Δ1* *rec104-Δ1*	Galbraith *et al.* (1997)
K65-m4	Isogenic to K65-3D except *mei4Δ::URA3* *mei4Δ::URA3*	Galbraith *et al.* (1997)
JN10-2-15B	Isogenic to K65-3D except *rad50Δ::URA3* *rad50Δ::URA3*	This article
JN10-2-54D	Isogenic to K65-3D except *rec104-Δ1 rad50Δ::URA3* *rec104-Δ1 rad50Δ::URA3*	This article
JK5-1-5D	Isogenic to K65-3D except *rec102-Δ2::LYS2* *rec102-Δ2::LYS2*	This article
JK5-1-1B	Isogenic to K65-3D except *rec104-Δ1 rec102-Δ2::LYS2* *rec104-Δ1 rec102-Δ2::LYS2*	This article
JK5-1-1A	Isogenic to K65-3D except *rec102-Δ2::LYS2 mei4Δ::URA3* *rec102-Δ2::LYS2 mei4Δ::URA3*	This article
JK8-7	Isogenic to K65-3D except *MAT*α*-inc-TRP5 lys2Δ::kan^r::pSPO13-HO* *MAT***a** *lys2-1*	This article
JK8-5	Isogenic to JK8-7 except *mei4Δ::URA3* *mei4Δ::URA3*	This article
JK8-8	Isogenic to JK8-7 except *rec104-Δ1* *rec104-Δ1*	This article
JK8-12	*MAT*α *mei4Δ::URA3 rad50S::URA3* *MAT***a** *mei4Δ::URA3 rad50S::URA3*	This article
JK8-13	*MAT*α *rad50S::URA3* *MAT***a** *rad50S::URA3*	This article

almost all of the coding region. Strains built by transformation were confirmed by both Southern analysis and genetic tests. Strains built by crossing were confirmed by PCR and genetic tests. Wild-type (K65-3D), *rec104* (K65-104-4A), and *mei4* (K65-m4) strains are decribed in Galbraith *et al.* (1997). One-step gene replacement (Rothstein 1991) was performed to obtain *rad50*Δ and *rec104*Δ *rad50*Δ strains. The null *rad50* mutation was the *rad50Δ::URA3* allele from pRM62 (Malone *et al.* 1990). Also, the 5.7-kb *Bgl*II-*Nsi*I fragment from pJK27 was used to make strains containing *rec102-Δ2::LYS2* by one-step gene replacement (Rothstein 1991).

Two sequential one-step gene replacements were performed to obtain JK8-7. First, the 7.6-kb *Pvu*II-*Xho*I fragment from pRM285 was transformed into K65-3D to obtain the *MAT***a**/*MAT*α*-inc::TRP5* strain. This strain was then transformed with a 5.1-kb *lys2Δ::kan^r::pSPO13-HO* PCR fragment made using the PCR primers Ho#1 [5′-CACCGCATCATCCAAGGATAG-3′] and Ho#2 [5′-GTAACGATGAAGCTGAGGAG-3′]. Transformants were selected on YPD plus Geneticin plates (100 μg/ml Geneticin; Bethesda Research Laboratories, Gaithersburg, MD; Guldener *et al.* 1996). Two sequential one-step gene replacements were also performed to generate JK8-5 and JK8-8.

RM96-15A, a closely related congenic strain containing the *rad50S* allele (Bullard *et al.* 1996), was crossed with K65-Het104-mei4, a K65-3D derivative heterozygous for both *mei4* and *rec104* (Galbraith *et al.* 1997). The resulting diploid was dissected to obtain JK8-11-5B (*MAT***a** *mei4Δ::URA3 rad50S::URA3*), JK8-11-5C (*MAT*α *mei4Δ::URA3 rad50S::URA3*), JK8-11-2B (*MAT***a** *rad50S::URA3*), and JK8-11-1D (*MAT*α *rad50S::URA3*). JK8-11-5B was crossed with JK8-11-5C to get JK8-12; and JK8-11-2B was crossed with JK8-11-1D to get JK8-13. JK8-12 was used for measuring meiotic DSBs in *mei4* cells, and JK8-13 was used as the *MEI4* control.

Media, growth, and sporulation conditions: Media, growth, and sporulation conditions have been described previously (Galbraith *et al.* 1997). For each experiment, all cultures were grown in the same medium and were treated identically. At least one culture of a WT strain (K65-3D) and one culture of a *rec104* strain (K65-104-4A) were included as a normal and an early timing control. We note, as previously observed (Galbraith *et al.* 1997), that the exact kinetics of sporulation can vary slightly from one experiment to another, but the relative timing does not.

Staining nuclei with 4′,6-diamidino-2-phenylindole and classifying cells: Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) and cells were examined with a fluorescent microscope as described previously (Galbraith *et al.* 1997). For time points ≤2 hr, at least 400 cells were counted; for all subsequent time points at least 1000 cells were counted. Mononucleate cells include those that have not entered meiosis and those that have just begun the meiotic process but have not yet undergone a division. Binucleate cells consist of cells that have undergone the first meiotic division. We define tetranucleate cells as cells having three or four nuclei (*i.e.*, cells have undergone both the first and the second meiotic divisions). Not all tetranucleate cells go on to form mature asci, especially in EE mutants. Thus, in EE mutants, the final percentage of sporulation is always less than the percentage of tetranucleate cells.

DNA analysis: The physical examination of DSB DNA was described in Bullard *et al.* (1996). To detect DSBs at the *MAT* locus, DNA was digested with *Eco*RI, subjected to electrophoresis, transferred to Hy-Bond N (Amersham, Arlington Heights, IL), and probed with the 1.1-kb *Hin*dIII-*Bam*HI fragment isolated from pRM285. In every experiment using the *pSPO13-HO* construct, the presence of DSBs at the *MAT* locus

was verified by Southern analysis. To directly compare the appearance of the *HO*-induced DSB with a natural meiotic DSB, the filter used for measuring the *HO* DSBs in the JK8-7 (Rec^+) diploid was reprobed with the 122-bp *Eco*RI-*Hin*dIII fragment isolated from pRM9 (Malone *et al.* 1994) to detect DSBs at the *HIS2* hotspot (Bullard *et al.* 1996). To examine DSBs at the *THR4* hotspot (Wu and Lichten 1995), DNA was digested with *Bgl*II and probed with a 0.9-kb fragment upstream of the *THR4* gene. Primers THf1 (5′-TGCCCGAT GATAAGGTCTCC-3′) and THr1 (5′-ATGTCACTCGTTCTT GCAGC-3′) were used to obtain the probe by PCR.

All imaging and quantification analysis was done using a Molecular Dynamics (Sunnyvale, CA) PhosphorImager Model 445SI as per instructions from the manufacturer.

Measurement of commitment to meiotic recombination at the *MAT* locus: Commitment to meiotic recombination was measured by a return-to-growth assay (Resnick *et al.* 1986). Cells were removed from sporulation medium at various time points and plated on rich medium (YPD) to allow mitotic growth. At least 200 colonies from two independent cultures from each time point were tested to determine the frequency of α-mating cells.

RESULTS

***rad50* mutants have an earlier reductional division than *rec104* mutants:** Our earlier work indicated that some EE genes (*REC102*, *REC104*, and *REC114 vs. MEI4*) can be distinguished because null mutations confer different effects on the timing of the reductional division (Galbraith *et al.* 1997). Of the known EE genes, *RAD50* is perhaps the most heavily studied (*e.g.*, Malone 1983; Alani *et al.* 1990; Cao *et al.* 1990; Padmore *et al.* 1991; Raymond and Kleckner 1993; Johzuka and Ogawa 1995). To understand the role of *RAD50* (an EE gene that is *not* meiosis-specific) in the relationship between recombination and the first division, we examined the effect of a *rad50* null mutation on the timing of the first division. In each experiment (Figure 2, A and B) one culture of a *rad50* diploid, a *rec104* diploid (early timing control), and a WT diploid (normal timing control) were examined. *rad50* cells undergo reductional division ~2 hr earlier than WT cells and ~1 hr earlier than the *rec104* mutant. This becomes clearer in an expanded view of early time points (Figure 2C). To verify this new *rad50* phenotype, we simultaneously examined the kinetics of the first division in four additional independent *rad50* cultures (with WT and *rec104* controls). All four independent *rad50* cultures initiated the first division about an hour earlier than the *rec104* mutant (data not shown). We conclude that a *rad50* null mutant initiates reductional division even earlier than a *rec104* mutant. The two different phenotypes allowed us to examine epistatic interactions between the *rad50* and *rec104* mutations. The timing in the *rad50 rec104* double mutant is indistinguishable from that in the *rad50* single mutant (Figure 2, A and B). From these observations we conclude that *rad50* is epistatic to *rec104*.

In previous work, EE mutants exhibiting a more rapid reductional division still proceeded through the second division at the normal time (Galbraith *et al.* 1997). Figure 2D shows that (like *rec102*, *rec104*, and *rec114)* a *rad50* mutation does not alter the timing of the initiation of the second division. This is consistent with the idea that the timing of the second division is, to some extent, independent of the timing of the first division (Galbraith *et al.* 1997).

***rec102* mutants, like *rad50*, have an even earlier reductional division than *rec104* mutants:** The discovery that the *rad50* mutants have an even earlier reductional division than *rec104* mutants reminded us of previous work, which hinted that *rec102* mutants might also confer this phenotype (Galbraith *et al.* 1997). To determine if *rec102* mutants truly go through the reductional division more rapidly than *rec104* mutants, we examined *rec102* and *rec104* again in detail (Figure 3). In both experiments, *rec102* cells (JK5-1-5D) began the first division about 1/2 to 1 hr earlier than isogenic *rec104* cells (K65-104-4A). The kinetics of the reductional division in the *rec102 rec104* double mutant (JK5-1-1B) are almost identical to those in the *rec102* single mutant, which leads to the conclusion that *rec102*, like *rad50*, is epistatic to *rec104* (Figure 3).

***rec102* is epistatic to *mei4*:** Our previous data (Galbraith *et al.* 1997) indicated that the EE genes *REC104* and *MEI4* could be placed into different groups because mutations in the two genes conferred different phenotypes with respect to the timing of reductional division: *mei4* cells undergo the first meiotic division at the normal time in contrast to the earlier division in *rec104* mutants. The *rec104* mutation is epistatic to *mei4*, which suggests a model for the order of action of these genes (Figure 1). In that article we predicted that *all* EE mutants conferring an earlier reductional division should be epistatic to *mei4*. We tested this prediction by comparing the timing of the reductional division in the *rec102 mei4* double mutant with that in each single mutant (Figure 4). As we found previously, *mei4* cells (K65-m4) begin reductional division at a time indistinguishable from WT cells. The timing of the reductional division in the *rec102 mei4* double mutant (JK5-1-1A) is early and is indistinguishable from the timing in the *rec102* single mutant (JK5-1-5D). This result indicates that *rec102* is epistatic to *mei4*, consistent with the hypothesis that EE genes act in a linearly dependent pathway (see discussion).

***MEI4* is required for the formation of meiosis-specific DSBs:** Null mutations in the EE genes tested thus far [*SPO11* and *RAD50* (Cao *et al.* 1990); *XRS2* (Ivanov *et al.* 1992); *MRE11* (Johzuka and Ogawa 1995); *MER2* (Rockmill *et al.* 1995); *REC102 REC104*, and *REC114* (Bullard *et al.* 1996); *MRE2* (Nakagawa and Ogawa 1997)] all prevent meiotic DSBs. The published phenotypes of *mei4* mutants strongly suggest that a *mei4* mutation should prevent the formation of meiotic DSBs (Menees *et al.* 1992; Nag *et al.* 1995); however, this has not been reported. The JK8-12 diploid is homozygous for *mei4*Δ*::URA3* in a *rad50S* background. *rad50S* allows meiotic DSBs to accumulate (Alani *et al.* 1990; Cao *et*

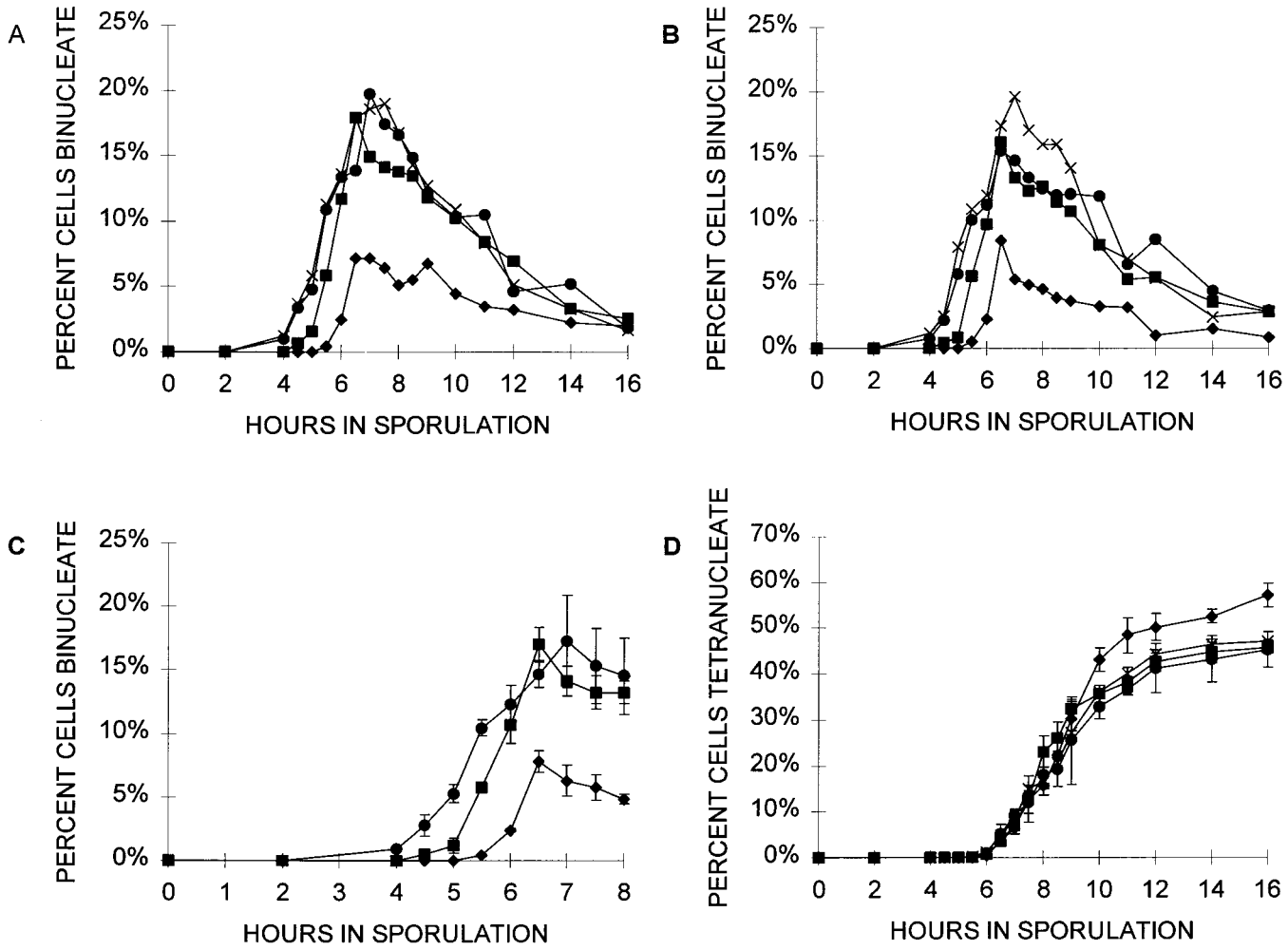

Figure 2.—Timing of the first and second division in *rad50* diploids. (A and B) Percentage of binucleate cells *vs.* time in two independent experiments. Isogenic WT (K65-3D; ◆), *rec104* (K65-104-4A; ■), *rad50* (JN10-2-15B; ●), and *rec104 rad50* (JN10-2-54D; ×) diploids were sporulated. Binucleate cells represent cells that have undergone the first meiotic division. Final sporulation for the WT, *rec104*, *rad50*, and *rec104 rad50* diploids averaged 74, 26, 31, and 31% mature asci, respectively. (C) An expanded view of the early times showing the standard deviations, from the two experiments in A and B. (D) Percentage of tetranucleate cells *vs.* time in sporulation. Tetranucleate cells represent cells having gone through both meiotic divisions. Data were averaged from the two independent experiments with error bars indicating the standard deviations.

al. 1990). No meiotic DSBs can be detected at the *THR4* hotspot (Goldway *et al.* 1993; Wu and Lichten 1994) in *mei4* mutant cells, whereas meiotic DSBs can be detected 3 hr after cells enter sporulation in *MEI4* control (Figure 5). Similar results were found at the *HIS2* hotspot (data not shown). This confirms that *MEI4* is required for the formation of meiosis-specific DSBs, and is consistent with our previous proposal that DSBs are not the signal for coordinating meiotic recombination and the reductional division (Galbraith *et al.* 1997; and see discussion).

The DSB induced by *pSPO13-HO* can induce recombination at the *MAT* locus in *mei4* and *rec104* mutants: The original model (Figure 1; Galbraith *et al.* 1997) proposed that the initiation of recombination creates a signal that normally delays the reductional division. Because a *mei4* diploid does not form meiotic DSBs, but enters the reductional division at a time indistinguishable from that of WT cells, DSBs do not appear to be the signal. However, although DSBs are not necessary for the normal delay, the presence of DSBs during prophase I might be recognized, communicate to the first division that recombination has started, and delay the first division. To test this possibility we introduced a DSB into EE mutant cells that normally have no breaks. Malkova *et al.* (1996) showed that the fusion of the *HO* endonuclease gene to the *SPO13* promoter can induce a DSB at the *MAT* locus during meiotic prophase I and that the kinetics of break formation by *HO* are indistinguishable to the kinetics of DSB formation at the *THR4* hotspot. We therefore used the *pSPO13-HO* construct to introduce a DSB in *rec104* and *mei4* mutants in early meiosis.

Southern blot analysis was performed to examine the appearance of the *pSPO13-HO*-induced DSBs at the *MAT* locus (Figure 6). Quantitative analysis confirms that the

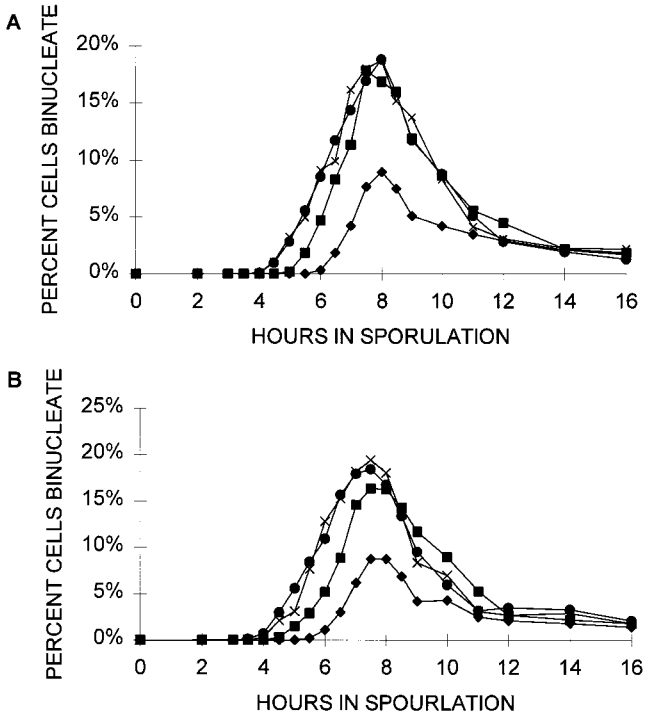

Figure 3.—(A and B) Percentage of binucleate cells *vs.* time in sporulation. A *rec102* diploid has an even earlier reductional division than a *rec104* diploid. Wild-type (K65-3D; ◆), *rec104* (K65-104-4A; ■), *rec102* (JK5-1-5D; ●), and *rec102 rec104* (JK5-1-1B; ×) diploids were sporulated. Samples were taken and stained as described in materials and methods. A and B represent two independent experiments. Final sporulation results (24 hr) for the WT, *rec104*, *rec102*, and *rec102 rec104* diploids averaged 82, 37, 35, and 36% mature asci, respectively.

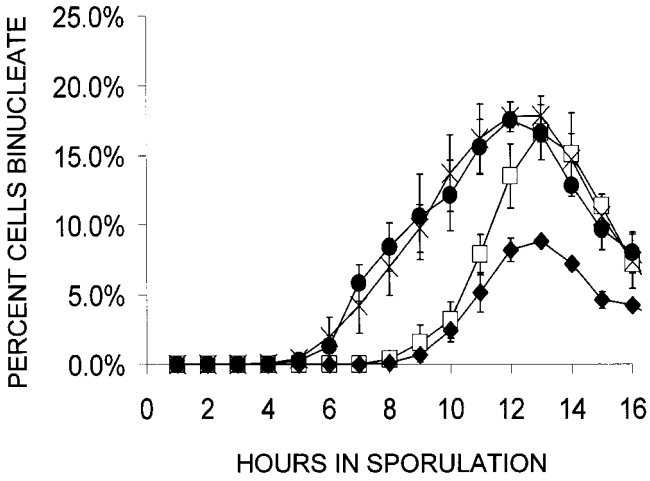

Figure 4.—A *rec102* mutation is epistatic to a *mei4* mutation. Isogenic WT (K65-3D; ◆), *mei4* (K65-m4; □), *rec102* (JK5-1-5D; ●), and *rec102mei4* (JK5-1-1A; ×) diploids were sporulated. Values shown are the means plus standard deviations of two independent cultures. Final sporulation results (24 hr) for the WT, *rec102*, *mei4*, and *rec102 mei4* diploids averaged 82, 37, 38, and 36% mature asci, respectively.

pSPO13-HO construct induces DSBs at the *MAT* locus in our strain background during the early stages of meiosis. Comparison with DSBs at the *HIS2* hotspot (Bullard *et al.* 1996) indicates that the *HO* DSBs appear at the same time as naturally occurring meiotic DSBs (Figure 6C). This result also demonstrates that DSBs created by *HO* can occur normally in an EE mutant [as found by Malkova *et al.* (1996) in their strain background].

Malkova *et al.* (1996) showed that the DSB generated by *pSPO13-HO* can induce meiotic recombination at the *MAT* locus in *rad50* cells, which are normally completely deficient in meiotic recombination. We asked if the *HO*-induced DSB could also induce recombination in *rec104* or *mei4* mutants. The *HO* DSBs could result in conversion of the *MAT***a** allele to the *MAT*α-*inc* allele, generating *MAT*α-*inc*/*MAT*α-*inc* diploids, which can be measured by mating tests (see materials and methods). The *pSPO13-HO* DSBs induce recombination at the *MAT* locus in both *rec104* and *mei4* diploids with a frequency and kinetics indistinguishable from a WT strain (Figure 6E). This result is consistent with the proposal that *REC104* and *MEI4* are not absolutely required for recombination after the formation of DSBs (see discussion).

The *HO*-induced DSB at the *MAT* locus has no effect on the timing of meiotic divisions in *mei4* and *rec104* mutants: As a control, we first tested the effect of the *HO* DSB on the timing of the reductional division in *mei4* cells. A comparison of the *mei4* mutant with and without the *pSPO13-HO* construct is shown in Figure 7. The timing of the first division is indistinguishable between the *mei4* diploid and the *mei4* diploid containing *pSPO13-HO*. Both strains initiate reductional division at about the same time as WT cells.

Unlike *mei4* cells, *rec104* cells start reductional division ~1 hr earlier than WT cells (Figures 2, 3, and Galbraith *et al.* 1997). We therefore tested whether a DSB induced by *pSPO13-HO* could restore normal timing of the reductional division in a *rec104* mutant. The timing

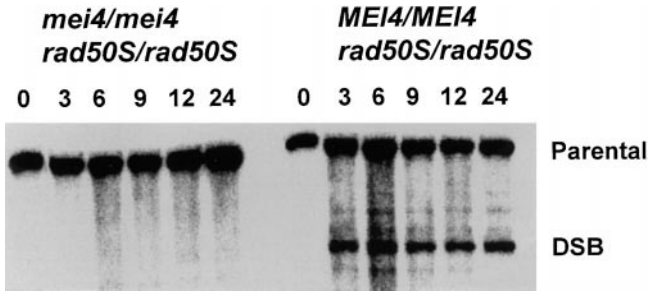

Figure 5.—*MEI4* is required for the formation of meiosis-specific DSBs. JK8-12 (*mei4*Δ*::URA3 rad50S::URA3*) and JK8-13 (*rad50S::URA3*) cells were sporulated and DNA was prepared from samples taken at various time points. Southern analysis was done by digestion of genomic yeast DNA with *Bgl*II and probing with the 0.9-kb fragment upstream of *THR4* (see materials and methods). This generates a 10.5-kb parental band and a 3.8-kb major meiotic DSB band.

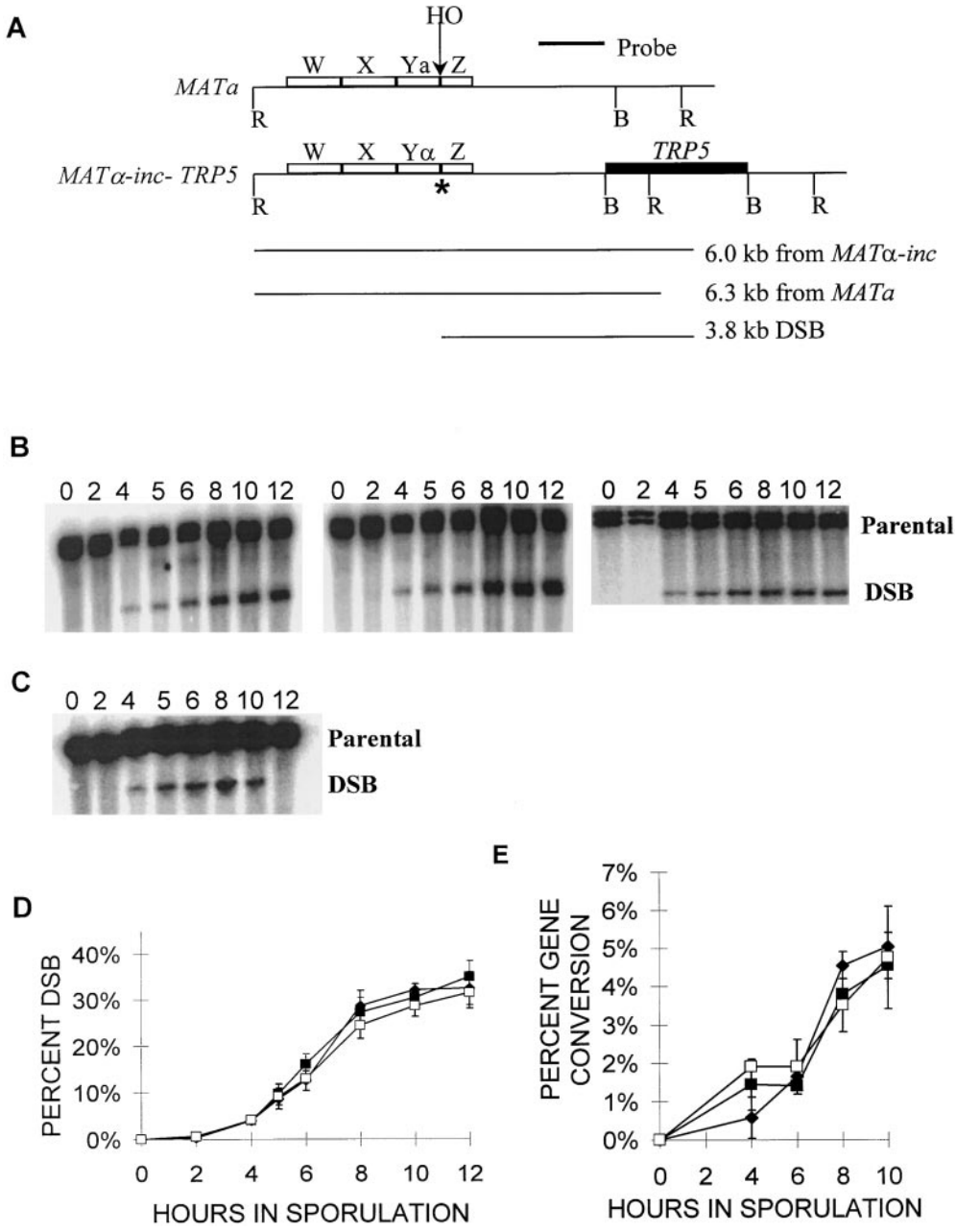

Figure 6.—Timing of *HO*-induced DSB formation at the *MAT* locus. (A) Structure of the *MAT* loci. After *Eco*RI digestion, the 1.5-kb *Hin*dIII-*Bam*HI probe (thick line) detects two parental bands (6.3 kb for *MAT***a** and 6.0 kb for *MATα-inc-TRP5*) and a 3.8-kb DSB band at the *MAT***a** locus caused by *HO*. The open boxes refer to the *MAT* loci (Astell *et al.* 1981), and the black box refers to the 2.8-kb *TRP5* gene. The asterisk indicates the α-*inc* mutation at the junction of Yα and Z region, which prevents digestion by *HO*. B, *Bam*HI; R, *Eco*RI. (B–E) Appearance of DSBs at the *MAT* locus. Two independent cultures of a WT [+*pSPO13-HO*] diploid (JK8-7; ◆), a *rec104* [+*pSPO13-HO*] diploid (JK8-8; ■), and a *mei4* [+*pSPO13-HO*] diploid (JK8-5; □) were sporulated. (Although only one culture is shown in B and C, both cultures were examined by Southern analysis and used to quantitate the results shown in D and E.) Numbers above the lanes in B and C refer to the number of hours the cells were in sporulation. The parental and the DSB bands are indicated. (C) The appearance of DSBs at the *HIS2* hotspot (Bullard *et al.* 1996). The same filter used in B for measuring *HO*-induced DSB in JK8-7 was reprobed with the 122-bp *Eco*RI-*Hin*dIII fragment upstream of the *HIS2* gene (see materials and methods). The parental and the DSB bands are indicated. (D) The fraction of total DNA in the DSB band was quantified as described in materials and methods and Bullard *et al.* (1996). (E) Commitment to gene conversion at the *MAT* locus induced by *pSPO13-HO*. The two cultures of WT [+*pSPO13-HO*] (JK8-7; ◆), *rec104* [+*pSPO13-HO*] (JK8-8; ■), and *mei4* [+*pSPO13-HO*] (JK8-5; □) diploids were examined by return-to-growth experiments for induction of recombination at the *MAT* locus (see materials and methods). At least 200 cells were examined for recombination at each time point for each culture to determine the percentage of α-mating cells resulting from a gene conversion event at the *MAT* locus.

of the reductional division in *rec104* cells containing *pSPO13-HO* is identical to that in *rec104* cells (Figure 7). From this experiment we conclude that the *pSPO13-HO*-induced DSB is not sufficient to restore normal timing of the first division in *rec104* cells.

We also examined the effect of the artificial *HO* DSB on the timing of the second meiotic division in *mei4* and *rec104* cells. The data show that there is no alteration of timing in *mei4* or *rec104* diploids (Figure 7, B and D), as we would have predicted from earlier observations and the model.

DISCUSSION

Epistatic interactions among the EE gene mutants: Our previous work (Galbraith *et al.* 1997) revealed one of the first phenotypic differences conferred by null EE mutations. Mutations in *REC102*, *REC104*, and

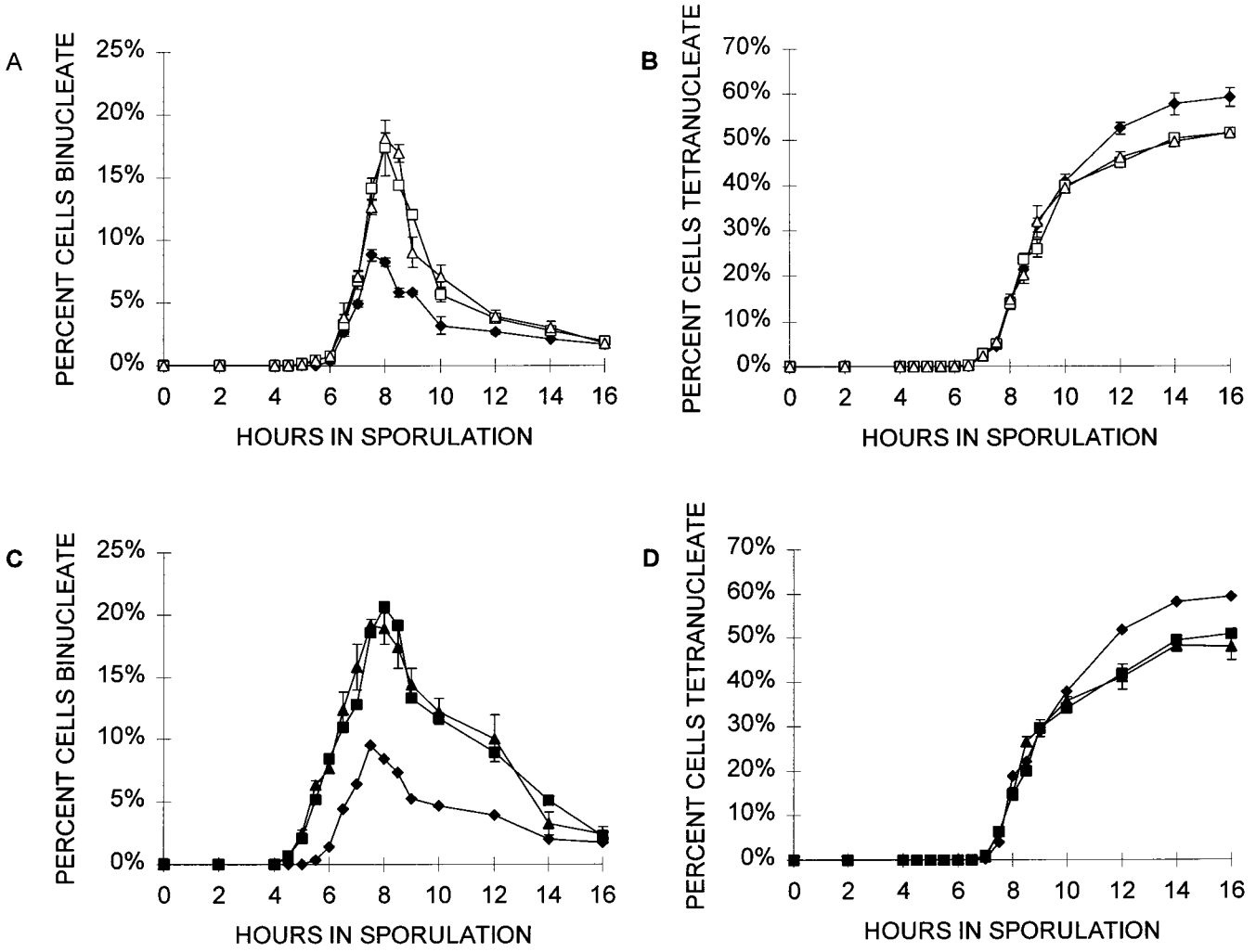

Figure 7.—The effect of the *HO*-induced DSB at the *MAT* locus on the timing of the meiotic divisions in *mei4* and *rec104* diploids. (A) Percentage of binucleate cells for two cultures of *mei4* [+*pSPO13-HO*] (JK8-5; △), WT (K65-3D; ◆), and *mei4* (K65-m4; □) diploids sporulated at the same time. (B) Percentage of tetranucleate cells *vs.* time in sporulation. Same symbols as in A. (C) Percentage of binucleate cells for two cultures of *rec104* [+*pSPO13-HO*] (JK8-8; ▲), *rec104* (K65-104-4A; ■), as well as a culture of WT (K65-3D; ◆); diploids all sporulated at the same time. (D) Percentage of tetranucleate cells *vs.* time in sporulation. Same symbols as in C.

REC114 confer an earlier reductional division than WT cells. In contrast, a *mei4* mutant undergoes the first division with timing indistinguishable from that of WT cells (Figure 4 and Galbraith *et al.* 1997). The work presented here adds *RAD50* to the group of EE genes required for normal timing of the reductional division. In addition, we observe a new phenotype in the *rad50* and *rec102* mutants: an even earlier reductional division than *rec104* mutants. The difference in timing allowed us to test epistatic interactions among the EE mutations. We have demonstrated that (1) *rec104* is epistatic to *mei4* (Galbraith *et al.* 1997), (2) *rec102* is epistatic to *mei4* (Figure 4), (3) *rad50* is epistatic to *rec104* (Figure 2), and (4) *rec102* is epistatic to *rec104* (Figure 3). These results are consistent with the hypothesis that EE genes, at least with respect to the timing of the first division, function in a linearly dependent pathway (see below).

In addition to the timing difference, another phenotypic difference among EE mutants was reported by Ohta *et al.* (1998). Mutations in four EE genes (*MRE11, RAD50, XRS2*, and *MRE2*) alter the micrococcal nuclease (MNase) sensitivity at a meiotic DSB site. The *mre2* and *mre11* mutants (type 1) confer a reduction in MNase sensitivity relative to WT cells, whereas the MNase sensitivity in *rad50* and *xrs2* mutants (type 2) reaches a higher level than the WT level. MNase sensitivity in the *mre11 rad50* double mutant is the same as that in the *mre11* single mutant, indicating that *mre11* (type 1) is epistatic to *rad50* (type 2) with respect to MNase sensitivity (Ohta *et al.* 1998). If the recombination pathway affects both chromatin structure (MNase sensitivity) and the timing of the first division in the same way, then we would predict that *mre11* should have a very early first division (*i.e.*, it should be upstream of *rad50*).

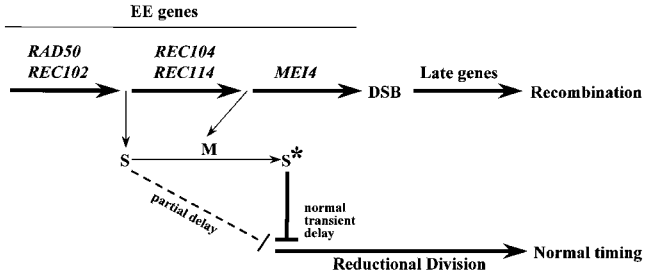

Figure 8.—A model for the interaction between initiation of recombination and reductional division. EE genes can be classified into three groups. Genes in the first group (*e.g.*, *RAD50*) can generate an initial signal, "S," which has partial activity to delay the reductional division. The genes in the second group (*e.g.*, *REC104*) result in the modification of S to "S*". S* is the fully functional signal responsible for the normal 2-hr transient delay of the reductional division. The genes in the third group like *MEI4* are not involved in the generation of the signal. Mutations in the first group of genes (*e.g.*, *RAD50*) eliminate the initial signal and therefore result in a very early reductional division. Mutations in the second group of genes (*e.g.*, *REC104*) cannot generate the modifier, "M", so no S* is made. However, the initial signal S is still made, thus leading a partial delay of the division. These mutants have a reductional division that is earlier than that of WT cells, but later than that of mutants in the first group. Mutations in the third group of genes (*e.g.*, *MEI4*) do not affect formation of the transient signal, and hence the initiation of reductional division is delayed as it should be and occurs at the normal time.

Different phenotypes conferred by EE mutations are examined in this article and in the work of Ohta *et al.* (1998). *RAD50* was the only gene examined in both studies. A null mutation in *RAD50* confers a very early first division and an increase in MNase sensitivity at a meiotic DSB site. It is possible that progressive alterations in chromatin structure are the signals for the timing of the reductional division. If this united view is true, one would also predict that *rec102* mutants should alter chromatin like *rad50* and that a *rec104* or *rec114* mutation might have different effects on chromatin structure than a *rad50* mutation. Finally, in this scheme, the meiotic chromatin of *mei4* mutants should appear like that from WT cells.

A revised model for communication between initiation of recombination and reductional division: Galbraith *et al.* (1997) proposed a model to explain the delay in reductional division caused by initiation of recombination. In this article we present data showing that *rec102* and *rad50* mutations confer a new phenotype, an even earlier first division than a *rec104* mutation. Furthermore, mutations in both *REC102* and *RAD50* are epistatic to a mutation in *REC104*. To account for this information, a revised model is presented in Figure 8. We propose that the EE genes may be sorted into three groups. The first group includes *RAD50* and *REC102*. Mutations in these genes result in a very early reductional division, ~2 hr earlier than division in WT cells. We propose that *RAD50* and *REC102* normally function to generate an initiation signal with partial ability to delay the first division. The second group would include *REC104* and *REC114*. Mutations in these genes cause the reductional division to occur ~1 hr earlier than it occurs in WT cells but ~1 hr later than in the *rec102* or *rad50* mutant. We propose that these genes would be required to alter the putative negative signal to the fully functional stage. As previously indicated (Galbraith *et al.* 1997), the signal must cause only a transient delay because WT cells do, of course, proceed through meiotic divisions. The third group of EE genes would include *MEI4*. Mutations in this last group of EE genes do not affect timing of the first division. We suppose that Mei4p acts after the formation of the signal. The fact that the EE$^-$ mutants fall into three classes suggests that the process that recognizes recombination initiation and leads to a delay in the first division is complex and that different steps in initiation may generate separate signals.

Our data indicate that recombination initiation is not needed for the first division (consistent with all published reports) and also that, in its absence, the first division can begin up to 2 hr earlier. In fact, in *rec102* or *rad50* cells, the first division begins at a time when recombination would normally be starting. This indicates that the first division apparatus is ready at the time of recombination, but is normally prevented from acting when recombination initiates. Padmore *et al.* (1991) found (in the SK1 background) that the first division normally occurs ~15 min after the completion of recombination. The transient 2-hr delay of the first division caused by the initiation of recombination makes sense because it would allow time for recombination to proceed before attempting to segregate chromosomes.

Meiotic DSBs do not serve as the transient signal for the delay of the first division: Both the original model (Figure 1; Galbraith *et al.* 1997) and the revised model in Figure 8 predict that meiotic DSBs are not the transient signal that normally delays the first meiotic division. This prediction is consistent with the phenotypes conferred by the *rad50S* alleles, which allow the accumulation of meiotic DSBs, but do not delay the first division or arrest cells through meiosis, although the *second* division is delayed in *rad50S* strains (Alani *et al.* 1990; Cao *et al.* 1990). This work shows that *MEI4* is required for the formation of meiotic DSBs. This result excludes meiotic recombination DSBs as a candidate for the transient signal, because *MEI4* is required for generating them, and yet *mei4* cells undergo reductional division with timing indistinguishable from that of WT cells.

Though meiotic DSBs cannot be the normal signal for delay of the first division, we wondered if a DSB occurring early in meiosis (or the recombination associated with it) *could* serve as a signal to delay the first division. It has been demonstrated in yeast that a single DSB can cause G2 arrest during mitosis, and one unre-

paired DSB even on a dispensable plasmid can lead to lethality (Bennett *et al.* 1993; Fairhead and Dujon 1993; Game 1993). Because mitotic cells appear to recognize a single DSB, the *pSPO13-HO*-induced break during meiotic prophase I might be recognized and restore normal timing in *rec104* cells. Such breaks should, however, have no effect on a *mei4* mutant.

To address this question, we used a *pSPO13-HO* construct to induce a DSB at the *MAT* locus during meiotic prophase I in *rec104* and *mei4* cells that normally have no DSBs. The percentage of DSBs created by the *HO* endonuclease reaches 30% of the total DNA by 12 hr. If one assumes that only one of the four chromatids is cut per cell, then we conclude that all the cells have a break by then ($4 \times 30\% \geq 100\%$). Previous results (Kolodkin *et al.* 1986; Malkova *et al.* 1996), however, suggest that it is more likely that both chromatids are often cut; in this case $\sim$60% of the cells have the break ($2 \times 30\%$). Because an average of 5–15% of the cells in our strains do not enter meiosis, we conclude that $\sim$70% of the cells passing through meiosis have the *HO* breaks. There is a degree of asynchrony in the population; not all cells move through meiosis at the same time and hence the *HO* break and the normal meiotic breaks occur over a period of several hours. We note that the kinetics of the induction of *HO* breaks in this experiment are the same as the kinetics of real recombination DSBs in the same WT cells. Further, the kinetics of the *HO* break in the Rec^- mutants are the same as in the WT Rec^+ cells. Because at least 60% of the cells contain the HO break, and because they follow the same kinetics as normal recombination breaks, we argue it would be possible to see the MI delay if it could be caused by the *HO* break.

The *HO* break did not affect the timing of the first division in *mei4* cells. More interestingly, the *HO* break did not restore normal timing of the first division in a strain (*rec104*) with an earlier first division. This result is consistent with the hypothesis that DSBs are not the transient signal arising from the initiation of recombination and indicates that the presence of one DSB during meiosis prophase I is not sufficient to institute the normal delay of reductional division. An alternative possibility, but one we think unlikely, is that a single *meiotic* DSB would be capable of delaying MI in a *rec104* strain, but that the *HO* break is not recognized by the delay system. This might result from the fact that the *HO* break can occur outside the context of normal recombination DSBs. For example, the HO break does not require the gene products of the meiosis-specific EE genes (*e.g.*, *REC104, MEI4, MER2*). Finally, we note that the *HO*-induced break appears (Figure 6) to persist over a somewhat longer period than the DSBs at *THR4* or *HIS2*. One interpretation is that the *HO* breaks are not processed with the same efficiency as normal meiotic recombination DSBs. An alternative interpretation is that the *HO* endonuclease is present for a longer time than the meiotic recombination initiation complex.

The transient signal proposed in Figure 8 could be a protein(s) or a post-translational modification of a protein(s). Alternatively, it could be an altered DNA or chromosomal structure (*e.g.*, see earlier discussion about meiotic chromatin), although it cannot be meiotic DSBs. Studies from different groups have shown that chromatin becomes more accessible at recombination hotspots before the formation of DSBs (*e.g.*, Ohta *et al.* 1994, 1998; Wu and Lichten 1994, 1995; Fan and Petes 1996). Using the technique of fluorescence in situ hybridization (FISH), Weiner and Kleckner (1994) demonstrated that homologous chromosomes associate with each other after premeiotic DNA synthesis prior to recombination. Consistent with the idea that homologs associate before meiotic DSB formation, examination of the *HIS2* hotspot indicates that a "hot" chromosome with increased DSBs can stimulate DSBs and recombination *in trans* on a "cold" homolog (Bullard *et al.* 1996). These authors argued that the best explanation was association or interaction of homologs prior to DSB formation. Xu and Kleckner (1995) found that a 32-bp *Bam*HI linker at the synthetic *HIS4::LEU2* hotspot also appeared to act *in trans* and argued that homologs must interact prior to the formation of DSBs. Though different quantitative results were obtained at the *HIS2* hotspot and the *HIS4::LEU2* hotspot, both observations agree with the proposal that homologs interact before meiotic DSBs are formed. This early "interacting" process between homologous chromosomes could be the basis of the transient signal to delay the first meiotic division.

Adding a DSB can induce gene conversion in a *mei4* or *rec104* mutant: Malkova *et al.* (1996) demonstrated that the *pSPO13-HO*-induced DSB can cause meiotic recombination in null *rad50* cells, which are normally deficient in meiotic recombination. The data in this article show that this construct can also induce gene conversion to the same level as that of the WT control in the other two EE mutants, *rec104* and *mei4*. Formally all of these results suggest that *RAD50*, *MEI4*, and *REC104* are only required before DSB formation. However, the break created by *HO* is different than the normal meiotic DSB. It is also possible that some genes required to make meiotic DSBs are needed after the breaks are formed. Certain recessive alleles of *RAD50* [*rad50S* alleles (Alani *et al.* 1990; Cao *et al.* 1990)] or *MRE11* [*mre11S* (Nairz and Klein 1997); *mre11-58* (Tsubouchi and Ogawa 1998)] cause a failure of DSB processing, allow the accumulation of DSBs, and confer the phenotype of a late exchange mutation. Thus, for the moment, we hesitate to completely exclude *MEI4* and *REC104* from all post-DSB meiotic roles.

Meiosis may be thought of as a well-controlled intracellular developmental process. High levels of meiotic recombination and the subsequent reductional division

are two of the unique events in meiosis. Our results demonstrate that several EE gene products required for the initiation of recombination are also involved in controlling the proper timing of reductional division, which suggests that these two unique meiotic processes do communicate with each other. The nature of the transient signal proposed in this article is under further investigation.

We thank James E. Haber for providing plasmids pL32 and pJH10. We thank Jan Fassler and Stuart Haring for reading and commenting on the paper and John Nau for technical assistance. This work was supported by National Institutes of Health grant R01-GM36846 to R.E.M.

LITERATURE CITED

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Astell, C. R., L. Ahlstrom-Jonasson, M. Smith, K. Tatchell, K. A. Nasmyth *et al.*, 1981 The sequence of the DNAs coding for the mating type loci of *Saccharomyces cerevisiae*. Cell **27:** 15–23.

Bennett, C. B., A. L. Lewis, K. K. Baldwin and M. A. Resnick, 1993 Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc. Natl. Acad. Sci. USA **90:** 5613–5617.

Berben, G., J. Dumont, V. Gilliquet, P. A. Bolleand and F. Hilger, 1991 The YDp plasmids: a uniform set of vectors bearing versatile gene disruption cassettes for *Saccharomyces cerevisiae*. Yeast **7:** 475–477.

Bhargava J., J. Engebrecht and G. S. Roeder, 1992 The *rec102* mutant of yeast is defective in meiotic recombination and chromosome synapsis. Genetics **130:** 59–69.

Bullard, S. A., S. Kim, A. M. Galbraith and R. E. Malone, 1996 Double strand breaks at the *HIS2* recombination hot spot in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **93:** 13054–13059.

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.

Cool, M., and R. E. Malone, 1992 Molecular and genetic analysis of the yeast early meiotic recombination genes *REC102* and *REC107/MER2*. Mol. Cell. Biol. **12:** 1248–1256.

Fairhead, C., and B. Dujon, 1993 Consequences of unique double-stranded breaks in yeast chromosomes: death or homozygosis. Mol. Gen. Genet. **240:** 170–178.

Fan, Q. Q., and T. D. Petes, 1996 Relationship between nuclease hypersensitive sites and meiotic recombination hot spot activity at the *HIS4* locus of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2037–2043.

Fan, Q. Q., F. Xu and T. D. Petes, 1995 Meiosis-specific double-strand DNA breaks at the *HIS4* recombination hot spot in the yeast *Saccharomyces cerevisiae*: control in *cis* and *trans*. Mol. Cell. Biol. **15:** 1679–1688.

Galbraith, A. M., and R. E. Malone, 1992 Characterization of *REC104*, a gene required for early meiotic recombination in the yeast *Saccharomyces cerevisiae*. Dev. Genet. **13:** 392–402.

Galbraith, A. M., S. A. Bullard, K. Jiao, J. J. Nau and R. E. Malone, 1997 Recombination and the progression of meiosis in *Saccharomyces cerevisiae*. Genetics **146:** 481–489.

Game, J. C., 1993 DNA double-strand breaks and the *RAD50-RAD57* gene in *Saccharomyces*. Semin. Cancer Biol. **4:** 73–83.

Guldener, U., S. Heck, T. Fiedler, J. Beinhauer and J. H. Hegemann, 1996 A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. **24:** 2519–2524.

Goldway, M., A. Sherman, D. Zenvirth, T. Arbel and G. Simchen, 1993 A short chromosomal region with major roles in yeast chromosome III meiotic disjunction, recombination and double strand breaks. Genetics **133:** 159–169.

Ippen-Ihler, K. A., and E. G. Minkley Jr., 1986 The conjugation system of F, the fertility factor of *Escherichia coli*. Annu. Rev. Genet. **2:** 593–624.

Ivanov, E. L., and J. E. Haber, 1997 DNA repair: RAD alert. Curr. Biol. **7:** R492–495.

Ivanov, E. L., V. G. Korolev and F. Fabre, 1992 *XRS2*, a DNA repair gene of *Saccharomyces cerevisiae*, is needed for meiotic recombination. Genetics **132:** 651–664.

Johzuka, K., and H. Ogawa, 1995 Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis specific double-strand-break formation in *Saccharomyces cerevisiae*. Genetics **139:** 1521–1532.

Kogoma, T., 1997 Stable DNA replication: interplay between DNA, homologous recombination, and transcription. Microbiol. Mol. Biol. Rev. **61:** 212–238.

Kolodkin, A. L., A. J. Klar and F. W. Stahl, 1986 Double-strand breaks can initiate meiotic recombination in *S. cerevisiae*. Cell **46:** 733–740.

Malkova, A., L. Ross, D. Dawson, M. F. Hoekstra and J. E. Haber, 1996 Meiotic recombination initiated by a double-strand break in *rad50*Δ yeast cells otherwise unable to initiate meiotic recombination. Genetics **143:** 741–754.

Malone, R. E., 1983 Multiple mutant analysis of recombination in yeast. Mol. Gen. Genet. **189:** 405–412.

Malone, R. E., S. Lin and J. Warning, 1990 The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr. Genet. **18:** 111–116.

Malone, R. E., S. Kim, S. A. Bullard, S. Lundquist, L. Hutchings-Crow *et al.*, 1994 Analysis of a recombination hot spot for gene conversion occurring at the *HIS2* gene of *Saccharomyces cerevisiae*. Genetics **137:** 5–18.

Mao-Draayer, Y., A. M. Galbraith, D. L. Pittman, M. Cool and R. E. Malone, 1996 Analysis of meiotic recombination pathways in the yeast *Saccharomyces cerevisiae*. Genetics **144:** 71–86.

Menees, T. M., P. B. Ross-MacDonald and G. S. Roeder, 1992 *MEI4*, a meiosis-specific yeast gene required for chromosome synapsis. Mol. Cell. Biol. **12:** 1340–1351.

Nag, D. K., H. Scherthan, B. Rockmill, J. Bhargava and G. S. Roeder, 1995 Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics **141:** 75–86.

Nairz, K., and F. Klein, 1997 *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. **11:** 2272–2290.

Nakagawa, T., and H. Ogawa, 1997 Involvement of the *MRE2* gene of yeast in formation of meiosis-specific double-strand breaks and crossover recombination through RNA splicing. Genes Cells **2:** 65–79.

Ohta, K., T. Shibata and A. Nicolas, 1994 Changes in chromatin structure at recombination initiation sites during yeast meiosis. EMBO J. **13:** 5754–5763.

Ohta, K., A. Nicolas, M. Furuse, A. Nabetani, H. Ogawa *et al.*, 1998 Mutations in the *MRE11, RAD50, XRS2*, and *MRE2* genes alter chromatin configuration at meiotic DNA double-stranded break sites in premeiotic and meiotic cells. Proc. Natl. Acad. Sci. USA **95:** 646–651.

Padmore, R., L. Cao and N. Kleckner, 1991 Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell **66:** 1239–1256.

Petes, T. D., R. E. Malone and L. S. Symington, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Cellular Biology of the Yeast Saccharomyces (Genome Dynamics, Protein Synthesis, and Energetics)*, edited by J. R. Broach, J. R. Ppringle and E. W. Jones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Pittman, D. L., W. Lu and R. E. Malone, 1993 Genetic and molecular analysis of *REC114*, an early meiotic recombination gene in yeast. Curr. Genet. **23:** 295–304.

Raymond, W. E., and N. Kleckner, 1993 RAD50 protein of *Saccharomyces cerevisiae* exhibits ATP-dependent DNA binding. Nucleic Acids Res. **21:** 3851–3856.

Resnick, M. A., J. Nitiss, C. Edwards and R. E. Malone, 1986 Meiosis can induce recombination in *rad52* mutants of *Saccharomyces cerevisiae*. Genetics **113:** 531–550.

Rockmill, B., J. A. Engebrecht, H. Scherthan, J. Loidl and G. S. Roeder, 1995 The yeast *MER2* gene is required for chromosome synapsis and the initiation of meiotic recombination. Genetics **141:** 49–59.

Rothstein, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast, pp. 281–301 in *Methods in Enzymology, Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. Fink. Academic Press, San Diego.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Sun, H., D. Treco and J. W. Szostak, 1991 Extensive 3′-overhanging, single stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **64:** 1155–1161.

Thaler, D. S., M. M. Stahl and F. W. Stahl, 1987 Tests of the double-strand-break repair model for Red-mediated recombination of phage lambda and plasmid lambda-dv. Genetics **116:** 501–512.

Tsubouchi, H., and H. Ogawa, 1998 A novel *mre11* mutation impairs processing of double-strand breaks of DNA during both mitosis and meiosis. Mol. Cell. Biol. **18:** 260–268.

Weiffenbach, B., D. T. Rogers, J. E. Haber, M. Zoller, D. W. Russell *et al.*, 1983 Deletions and single base pair changes in the yeast mating type locus that prevent homothallic mating type conversions. Proc. Natl. Acad. Sci. USA **80:** 3401–3405.

Weiner, B. M., and N. Kleckner, 1994 Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell **77:** 977–991.

Wu, T. C., and M. Lichten, 1994 Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science **263:** 515–518.

Wu, T. C., and M. Lichten, 1995 Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics **140:** 55–66.

Xu, L., and N. Kleckner, 1995 Sequence non-specific double-strand breaks and interhomolog interactions prior to double strand break formation at a meiotic recombination hot spot in yeast. EMBO J. **14:** 5115–5128.

Communicating editor: M. Lichten