Meiotic Recombination Initiated by a Double-Strand Break in *rad50Δ* Yeast Cells Otherwise Unable to Initiate Meiotic Recombination

A. Malkova,* L. Ross,[†,1] D. Dawson,[†] Merl F. Hoekstra[‡] and J. E. Haber*

**Rosenstiel Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254-9110, [†]Department of Microbiology, Tufts University, Boston, Massachusetts 02111 and [‡]ICOS Corporation, Bothell, Washington 98201*

Manuscript received December 29, 1995
Accepted for publication March 13, 1996

ABSTRACT

Meiotic recombination in *Saccharomyces cerevisiae* is initiated by double-strand breaks (DSBs). We have developed a system to compare the properties of meiotic DSBs with those created by the site-specific HO endonuclease. HO endonuclease was expressed under the control of the meiotic-specific *SPO13* promoter, creating a DSB at a single site on one of yeast's 16 chromosomes. In Rad$^+$ strains the times of appearance of the HO-induced DSBs and of subsequent recombinants are coincident with those induced by normal meiotic DSBs. Physical monitoring of DNA showed that *SPO13::HO* induced gene conversions both in Rad$^+$ and in *rad50Δ* cells that cannot initiate normal meiotic DSBs. We find that the *RAD50* gene is important, but not essential, for recombination even after a DSB has been created in a meiotic cell. In *rad50Δ* cells, some DSBs are not repaired until a broken chromosome has been packaged into a spore and is subsequently germinated. This suggests that a broken chromosome does not signal an arrest of progression through meiosis. The recombination defect in *rad50Δ* diploids is not, however, meiotic specific, as mitotic *rad50* diploids, experiencing an HO-induced DSB, exhibit similar departures from wild-type recombination.

STUDIES in *Saccharomyces cerevisiae* have shown that double-strand breaks (DSBs) are efficient initiators of homologous recombination in both mitotic and meiotic cells. Both mitotic DSBs, initiated by the site-specific endonuclease HO, or meiotic DSBs are initially processed in similar ways, yielding 3′ single-stranded regions adjacent to the break (reviewed by HABER 1995). Subsequent recombination appears to follow the scheme set out by RESNICK and MARTIN (1976) and by SZOSTAK *et al.* (1983), although the proportion of gene conversion events that are resolved with an accompanying crossover is generally greater in meiosis than in mitosis. However, it is not clear if these mitotic and meiotic recombination events are in fact equivalent. Part of the problem in comparing mitotic and meiotic DSBs is that some of the genes that are important, but not essential, in mitotic recombination appear to play very different, and essential, roles in the creation of meiotic DSBs. For example, a deletion of either the *RAD50* or *XRS2* genes makes cells hyper-recombinational for spontaneous mitotic recombination (MALONE and ESPOSITO 1981; MALONE *et al.* 1990; IVANOV *et al.* 1992) and causes a delay in the kinetics of homologous recombination initiated by HO endonuclease (SUGAWARA and HABER 1992; IVANOV *et al.* 1994). In contrast, the absence of these same genes abolishes the creation of meiotic DSBs and eliminates recombination (MALONE and ESPOSITO 1981; MALONE 1983; BORTS *et al.* 1986; CAO *et al.* 1990; IVANOV *et al.* 1992); hence, the roles of *RAD50* and *XRS2* during later steps in meiosis are difficult to assess.

Recently a special allele, *rad50S* (ALANI *et al.* 1990), has been used in meiotic studies because it allows the creation of DSBs but prevents subsequent 5′ to 3′ exonuclease degradation of the DNA ends. These studies showed that meiotic DSBs are unusual, at least under *rad50S* mutant conditions. A protein is attached to the 5′ ends of the DSB, which are either blunt ends or have short 5′ overhangs (DE MASSY *et al.* 1995; KEENEY and KLECKNER 1995; LIU *et al.* 1995). The presence of this protein apparently prevents the normal, extensive 5′ to 3′ exonucleolytic digestion of the cut ends (SUN *et al.* 1991). This type of degradation to produce long 3′-ended single-stranded DNA tails is very similar to that observed after mitotic cleavage of DNA by HO (WHITE and HABER 1990; SUGAWARA and HABER 1992). The blunt or 5′-protruding nature of meiotic DSBs distinguishes them from those created by the HO or I-SceI endonucleases in mitotic cells, which have 3′ overhangs (KOSTRIKEN *et al.* 1983; COLLEAUX *et al.* 1988).

The formation of DSBs is part of a complex series of events in meiosis. Current understanding of meiosis does not yet allow us to establish fully the causal relationship between DSB formation, the search for homologous DNA sequences, the alignment of homologous chromosomes, the formation of the synaptonemal complex and the regulation of crossing over. Some experi-

Corresponding author: James E. Haber, Rosenstiel Center, Mailstop 029, Brandeis University, Waltham, MA 02254-9110.
E-mail: haber@hydra.rose.brandeis.edu

[1] *Present address:* Department of Molecular and Human Genetics, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030-3498.

ments suggest that interactions between homologous regions occur before the initiation of DSBs, whose frequency might even be regulated by this prior pairing (SCHERTHAN *et al.* 1992; WEINER and KLECKNER 1994; XU and KLECKNER 1995). However, in the absence of meiotic DSBs there is no recombination, the synaptonemal complex does not form between homologues, and there is massive chromosome nondisjunction.

Viable spores can be recovered in the absence of meiotic crossing over in diploids deleted for *SPO13* (MALONE and ESPOSITO 1981; KLAPHOLZ *et al.* 1985). Under these circumstances all chromosomes undergo a single equational division to produce two spores, each with the phenotype of the original diploid strain. However in recombinationally proficient *spo13*Δ diploids, some chromosomes undergo a single reductional division (HUGERAT and SIMCHEN 1993). The frequency with which a chromosome exhibits a reductional division appears to depend on sequences at or near the centromere but might also depend on the location and frequency of crossover events. It is not known if a single crossover event on a chromosome would be sufficient to cause a reductional division in *spo13* diploids.

To assess how these steps are related, it is important to determine if DSBs created in an otherwise recombinationless meiotic cell would restore the normal sequence of events in meiosis, including normal chromosome segregation. An indication that this might be so comes from the work of THORNE and BYERS (1993), who demonstrated that ionizing radiation could partially restore normal chromosome disjunction in a *spo11* diploid that also has no meiotic DSBs. To address these questions, we have expressed the HO endonuclease in meiotic cells, under the control of the meiotic-specific promoter of the *SPO13* gene. We find that there are some important differences in the creation of HO-initiated meiotic DSBs compared to normal meiotic DSBs, but that in general recombination appears to be similar. We show that the *RAD50* and *XRS2* genes are important, but not essential, for recombination even after a DSB has been created in a meiotic cell.

MATERIALS AND METHODS

Plasmids and gene modification: The *SPO13::HO* fusion was prepared in seven steps. The *SPO13* promoter region (−160 to −3) was PCR amplified and cloned into the *Cla*I and *Sal*I sites in the polylinker of Bluescript. A PCR-amplified fragment corresponding to the coding sequence of the HO endonuclease gene was then cloned downstream of the *SPO13* promoter. To allow integration of the *SPO13::HO* construct into a chromosome, we integrated the *SPO13::HO* fusion into the *LYS2* gene on plasmid YIp333 (EIBEL and PHILIPPSEN 1983), substituting the central part of *LYS2* gene. In the resulting plasmids pL30 and pL32, the *SPO13::HO* construct is inserted in the *LYS2* gene in opposite orientations. An analogous *SPO11::HO* fusion gene was inactive (M. F. HOEKSTRA, unpublished observation).

pFH800 contained a *GAL10::HO* fusion cloned into an *ARS1 CEN4 TRP1* vector (NICKOLOFF *et al.* 1986) and was used to induce HO DSBs in mitotically growing yeast cultures.

Strains: Genotypes of the yeast strains are listed in Table 1. The DL strains used in the gene conversion experiments in Table 2 are all derived from strains YEF62 (*MATα-inc leu2 ura3 his3 trp1 lys2*) and YEF71 (*MAT***a** *leu2 ura3 his3 trp1 lys2*) obtained from E. FOSS and F. STAHL. These were backcrossed three or more times with isogenic derivatives of strain MGA (NICOLAS *et al.* 1989). Halpoid derivatives were transformed with the *SPO13::HO* gene. Strain DAM1 was derived from cross between strain G293 (*MATα ade1 his4 trp1 leu2 ura3*) and DL 1029 (*MAT***a***-inc leu2 his3 trp1 ura3 lys2::SPO13::HO arg4*), a haploid parent of the DL strains. All DAM strains inherited the *SPO13::HO* construct from segregant of this cross. Dipoid strains DAM59, DAM60, DAM62, DAM65, DAM68, DAM76, DAM77, DAM94, DAM95, DAM98 are isogenic to each other and were derived by crossing strains AM58 (*MATα-inc ade1 lys2::SPO13::HO met13-2 leu2-3,112 trp1 thr4*) and AM67 (*MAT***a** *ade1 lys2::SPO13::HO met13-2 leu2-3,112 trp1*). Strain DAM15 was obtained from a cross between a *his4* derivative of AM58 and A808 (*MATα ade1 his4-519 ura3-52 leu2-3,112*). Strain DAM73 was derived from cross of AM58 and DAM15 segregant 1D. Strain DAM75 was derived from backcross of DAM15 segregant 4C to a *spo13 rad50* derivative of AM58. Strain DAM97 was derived from a cross of DAM75 segregant 53C and AM181 (*MATα-inc ade1 lys2::SPO13::HO met13-2 leu2 thr4 tsm1*). The nonisogenic DL and DAM strains were used in the experiments described in Table 2 to demonstrate that *SPO13::HO*-induced events were similar in various strain backgrounds. In all of the other experiments, comparisons were made between pairs of isogenic or congenic strains.

Deletion-disruption alleles of *RAD50, XRS2* and *SPO13* were constructed by the one-step gene disruption method (ROTHSTEIN 1983). To disrupt *XRS2*, plasmid pEI40 (IVANOV *et al.* 1994) was digested with *Hin*dIII and used to transform recipient strains to Leu$^+$. To disrupt *RAD50*, plasmid pNKY83 (ALANI *et al.* 1989) was digested with *Bgl*II and *Eco*RI and used to transform recipient strains to Ura$^+$ (*rad50::hisG::URA3::hisG*). To disrupt *SPO13*, plasmid pNKY58 (obtained from N. KLECKNER) was digested with *Bam*HI and used to transform recipient cells to Ura$^+$ (*spo13::hisG::URA3::hisG*). When necessary, Ura$^-$ derivatives (*rad50::hisG* and *spo13::hisG*) were selected on 5-fluoro-orotic acid (5-FOA) plates (BOEKE *et al.* 1984). To introduce the *LYS2::SPO13::HO* construct into the chromosome, plasmids pL30 or pL32 bearing the *LYS2::SPO13::HO* construct were digested with *Hpa*I and *Nhe*I. These digests were used to introduce the *SPO13::HO* into the chromosome by one-step gene transplacement. Transformants were selected on α-aminoadipate plates (CHATTOO *et al.* 1979). To introduce the *ADE1* gene into the *HML* locus plasmid pJH1179 (X. WU and J. E. HABER, unpublished results) was digested with *Bam*HI and used to transform recipient strains to Ade$^+$. To introduce the *URA3* gene into the chromosome *III* region 10 kb proximal to the *MAT* locus, plasmid pJH106 (KRAMER and HABER 1993) was digested with *Bam*HI and used to transform recipient strains to Ura$^+$. To introduce the *URA3* gene into the *BUD5* locus, the pAF228 plasmid (THIERRY *et al.* 1990) was digested with *Xmn*I and used to transform recipient strains to Ura$^+$. The *arg4-Bgl*II and *arg4-Eco*RV mutations were integrated in the genome as follows. Plasmids pNPS309 and pNPS314 (a gift from NEIL SCHULTES and JACK SZOSTAK) bearing these *arg4* mutant alleles plus the *URA3* gene (NICOLAS *et al.* 1989) were digested with *Eco*47III and integrated in the *ARG4* chromosomal gene of recipient strains using selection to Ura$^+$. Then Ura$^-$ derivatives were selected on 5-FOA plates. Some of these Ura$^-$ colonies were Arg$^-$ variants and retained the *arg4* mutant allele in the chromosome. All strain constructions were verified by Southern blot hybridization. The *rad50S* mutation was introduced into the DL strains (Table 1) by crossing haploid *rad50S* strains of similar genetic background, provided by ALAIN NICOLAS (DE MASSY and NICOLAS 1993).

TABLE 1

Yeast strains used in this study

Strain	Genotype
DAM15	*MATa/MATα-inc ade1-100/ade1 his4-519/his4'-URA3-HOcsLEU2HOcs'-his4 LYS2/lys2::SPO13HO ura3-52/ura3 leu2-3,112/leu2-3,112 trp1/TRP1*
DAM73	*MATa/MATα-inc ade1/ade1 HIS4/his4-519 lys2::SPO13::HO/lys2::SPO13::HO MET13/met13-2 ura3/ura3 leu2-3,112/leu2-3,112 trp1/trp1 THR4/thr4 arg4-BglII/arg4-EcoRV*
DAM75	*MATa/MATα-inc ade1/ade1 lys2::SPO13::HO/LYS2 met13-2/met13-2 ura3/ura3 leu2-3,112/leu2 trp1/trp1 rad50Δ/RAD50 spo13Δ/SPO13*
DAM97	*MATa/MATα-inc ade1/ade1 lys2::SPO13::HO/lys2::SPO13::HO met13-2/met13-2 leu2/leu2 trp1/TRP1 THR4/thr4 tsm1/TSM1*
DAM1	*MATa-inc/MATα HIS4/his4'-URA3-HOcsLEU2HOcs'-his4 HIS3/his3 ura3/ura3 leu2/leu2 trp1/trp1 LYS2/lys2::SPO13::HO ADE1/ade1*
DAM59	*MATa/MATα-inc ade1/ade1 lys2::SPO13::HO/lys2::SPO13::HO met13-2/met13-2 ura3/ura3 leu2-3,112/leu2-3,112 trp1/trp1 thr4/THR4 rad50Δ/rad50Δ spo13Δ/spo13Δ*
DAM60	DAM59, but *MATprox.::URA3/MATprox.*
DAM62	DAM59, but *LYS2/LYS2*
DAM65	DAM59, but *RAD50/RAD50 xrs2Δ/xrs2Δ*
DAM68	DAM60, but *hml:ADE1/HML*
DAM77	DAM68, but *hmr::LEU2/HMR*
DAM98	DAM68, but *RAD50/rad50Δ hml::ADE1/hml::LEU2*
DAM76	DAM68, but *LYS2/LYS2*
DAM95	DAM59, but *bud5::URA3/BUD5*
DAM94	DAM95, but *RAD50/RAD50*
DL1001	*MATa/MATα-inc leu2/leu2 trp1/trp1 his3/HIS3 ura3/ura3 lys2/LYS2 arg4/ARG4*
DL1003	*MATa/MATα-inc leu2/leu2 trp1/trp1 his3/his3 ura3/ura3 lys2/LYS2 arg4/ARG4*
DL1015	*MATa/MATα-inc leu2/leu2 trp1/trp1 his3/his3 ura3/ura3 lys2/LYS2 arg4/ARG4*
DL1023	*MATa-inc/MATα leu2/leu2 trp1/trp1 his3/HIS3 ura3/ura3 lys2::SPO13::HO/LYS2 lys1/LYS1 arg4/ARG4*
DL1024	*MATa-inc/MATα leu2/leu2 trp1/trp1 his3/HIS3 ura3/ura3 lys2::SPO13::HO/LYS2 lys1/LYS1 arg4/ARG4*
DL1042	*MATa-inc/MATα leu2/leu2 trp1/trp1 his3/HIS3 ura3/ura3 lys2::SPO13::HO/LYS2 lys1/LYS1 arg4/arg4*
DL645	*MATa/MATα leu2-3,112/leu2-3,112 trp1-289/trp1-289 ura3-52/ura3-52 his3-11,15/his3-11,15 ade1/ADE1 arg4-ΔHpaI/arg4-ΔHpaI lys2::SPO13::HO/lys2::SPO13::HO rad50S::URA3/rad50S::URA3*

Growth and sporulation conditions: Rich medium (YEPD), synthetic complete medium (MA) with bases and amino acids omitted as specified were as described (KAISER *et al.* 1994). Presporulation medium (YPA) was YEPD with potassium acetate substituted for glucose (MALONE *et al.* 1991). Sporulation medium (SM) contained 2% potassium acetate and was supplemented with appropriate nutritional ingredients (KAISER *et al.* 1994). Inheritance of the Rad^- phenotype was followed by the inability of cells to grow on YEPD plates with 0.015% MMS purchased from Sigma. YEP medium was YEPD without glucose. YEPG and YEPgal medium were YEP with 3% glycerol or 2% galactose substituted for glucose, respectively.

Sporulation of mass cultures: Saturated overnight cultures in YEPD media were diluted 1/1000 into 500 ml of YPA media and grown with vigorous shaking to $2–5 \times 10^7$ cells per ml. Cells were harvested by centrifugation, washed once with 1% potassium acetate, resuspended at $\sim 2 \times 10^7$ cells per ml in 500 ml of SM and aerated with vigorous shaking at 30° in a 2-l flask. Samples were removed at intervals for extraction of DNA and for study of commitment to recombination. Yeast genomic DNA was purified according to protocol described by GOYON and LICHTEN (1993) and digested for Southern blots with appropriate enzymes.

Commitment to meiotic recombination: Culture aliquots were sonicated briefly to disrupt clumps, diluted and plated on synthetic medium lacking arginine to select Arg^+ recombinants and onto YEPD to measure total viable cells and to score α-mating diploids. Commitment to meiotic recombination was measured by scoring Arg^+ prototrophs in return-to-growth experiments with the diploid DAM73 bearing *arg4-BglII/arg4-EcoRV* heteroalleles. Commitment to *SPO13::HO*-induced recombination was measured by scoring the α-mating cells appearing in the same experiment; these result from gene conversion of *MATa* by *MATα-inc* in this *MATa/MATα-inc* (nonmating) diploid.

Analysis of DNA: Standard techniques were used for restriction enzyme digestion, agarose gel electrophoresis, and Southern blot analysis (SAMBROOK *et al.* 1989). Densitometry of autoradiograms was carried out by using a Molecular Dynamics Storage PhosphorImager.

Galactose induction of HO: HO-mediated recombination at the *MAT* locus was induced in mitotically growing cell cultures as described (WHITE and HABER 1990). Briefly, cells were pregrown in liquid MA medium selective for the plasmid bearing the *GAL::HO* construct. Cultures were transferred to YEPG and grown overnight to a cell density of 1×10^7 cells per ml. Cells were collected by centrifugation, washed with YEP media and resuspended in YEPgal at a cell density 1×10^7 cells per ml. After 2 hr of incubation in YEPgal, cells were diluted in water and plated on YEPD plates.

Genetic analysis: Standard methods were used for ascus dissection, crosses, and other genetic procedures (KAISER *et al.* 1994).

Analysis of linkage of markers on chromosome *III* in *rad50*

diploids: *rad50* diploids show an elevated, though weak, level of chromosome loss. As a consequence we were able to make use of a strategy that we previously used to analyze the linkage of markers on chromosome *III* in a *rad52* diploid, where chromosome losses also occur (HABER and HEARN 1985). We used a diploid such as DAM68, which is heterozygous for *URA3* inserted on the right arm of chromosome *III* and proximal to *MAT*. By plating cells on 5-FOA-containing medium, we could recover Ura$^-$ colonies, many of which had lost both the *hml::ADE1* marker on the left arm of the chromosome and both *MAT***a** and the more distal *THR4* allele. This allowed the analysis of diploid segregants, exhibiting a parental phenotype, to determine if there had been "hidden" crossover events. The elevated chromosome loss of *rad50* diploids also allowed us to detect diploids containing a *MAT/HMR***a** fusion, a haplo-lethal deletion of ~100 kb of the right arm of the chromosome (HAWTHORNE 1963). In normal *rad50* diploids, chromosome loss yields $2n - 1$ *MAT***a** or $2n - 1$ *MATα-inc* aneuploids to give weak mating with both *MAT***a** and *MATα* tester strains. Diploids containing a recessive lethal *MAT/HMR***a** deletion and *MATα-inc* only exhibit α-mating.

RESULTS

SPO13::HO*-induced DSBs occur at the time of meiotic DSBs:** In mitotic cells, recombination induced by the site-specific endonuclease HO has been well studied (HABER 1995). We wished to develop a system by which we could compare the way recombination occurs in meiotic and mitotic cells, initiated by the same DSB. This can be accomplished by using a gene fusion of HO to the *SPO13* promoter. Transcription of the *SPO13* gene is induced early in meiotic prophase at roughly the same time as meiotic DSBs occur (WANG *et al.* 1987; BUCKINGHAM *et al.* 1990; ZENVIRTH *et al.* 1992). A *SPO13::HO* gene was integrated into the yeast genome in the *LYS2* locus (see MATERIALS AND METHODS). To demonstrate that *SPO13::HO*-driven DSBs occurred at the proper time in meiotic cells, we compared the creation of HO-induced cleavages of the *MATa** locus with the formation of normal meiotic DSBs at the previously characterized "hot spot" located in the *THR4* proximal region (ZENVIRTH *et al.* 1992; WU and LICHTEN 1994) using Southern blot analysis. In these experiments diploid DL645 homozygous for the *rad50S* mutation was employed to prevent processing of meiotic DSBs (ALANI *et al.* 1990). DSBs at *MAT* and at the *THR4*-adjacent hot spot begin to accumulate at approximately the same time (Figure 1, A and B). Isogenic strains without the *SPO13::HO* construct did not experience breaks at the *MAT* locus (not shown).

The similarity of timing of normal meiotic recombination and *SPO13::HO*-induced events can also be demonstrated in Rad$^+$ cells. We constructed a *MAT***a**/*MATα-inc arg4-Eco*RV/*arg4-Bgl*II diploid (strain DAM73) in which *MAT***a** can be cleaved by HO but the *MATα-inc* locus cannot. The time of commitment to recombination during meiosis can be assessed by measuring recombination after removing cells from the nitrogen-free medium that supports meiosis and plating them on growth medium (SHERMAN and ROMAN 1963). Recombination at *MAT* can be followed by the conversion of the nonmating (*MAT***a**/*MATα-inc*) diploid to α-mating (*MATα-inc*/*MATα-inc*). The time of appearance of *MAT* recombinants was coincident with the kinetics of appearance of Arg$^+$ recombinants (Figure 2).

Meiotic-like gene conversions are induced by *SPO13::HO*: *SPO13::HO*-induced recombination was analyzed in the meiotic progeny of *MAT***a**/*MATα-inc* and *MAT***a**-*inc*/*MATα* diploids (Table 2). In these diploids the HO-produced DSBs could be introduced only at one copy of the *MAT* locus, because the other one contained either an **a**-*inc* or *α-inc* mutation, interrupting the HO cut site (WEIFFENBACH *et al.* 1983). In the case of *MAT***a**/*MATα-inc* diploids, the repair of these DSBs by gene conversion resulted in the appearance of tetrads containing "extra" α-mating spores: 3α:1**a** (conversion occurring in one chromatid) or 4α:0**a** (conversion occurring in two chromatids) in the case of *MAT***a**/*MATα-inc* diploid. In the case of *MAT***a**-*inc*/*MATα* diploid, tetrads with extra **a**-mating spores were formed as a result of DSB repair.

For several diploids analyzed, from 10 to 31% of all tetrads exhibited gene conversion at *MAT*. Among them, the percentage of 3:1 events varied from 13 to 50% of all conversions. These variations in frequencies of gene conversion probably reflect variations in expression of *SPO13::HO* depending on strain background, orientation of *SPO13::HO* construction and number of copies of *SPO13::HO*. In a previous attempt to use HO to initiate events in meiotic cells, a galactose-inducible *HO* gene was used (KOLODKIN *et al.* 1986); either the timing of *HO* expression or the amount of HO cleavage yielded only tetrads with 4α:0**a** spores. Based on the gene conversion data alone, the events reported by KOLODKIN *et al.* (1986) could not be definitively concluded to have occurred after premeiotic DNA replication. In the case of *SPO13::HO*, we can conclude that the 3α:1**a** and 1α:3**a** events certainly occurred after premeiotic DNA replication. We believe that expression of HO is not equal in the population of meiotic cells, so that some cells have sufficient HO to cleave both *MATα* chromatids and produce 4:0 tetrads. Probably most of the 4:0 tetrads also arose after DNA replication, when there was enough HO endonuclease expressed to cleave more than one target. It is also possible that the paucity of 3:1 events stems from repair of one HO-cut chromatid by its sister chromatid, as suggested by KOLODKIN *et al.* (1986).

Southern blot analyses performed on the α-spores arising from four 3α:1**a** and 16 4α:0**a** tetrads indicated that, in every case, DSB repair occurred by using *MATα-inc* located on the homologous chromosome as the donor, even though the strains also carry the silent copy *HMLα* donor. We distinguished recombination with *MATα-inc* from recombination with *HMLα* by Southern blot, as *MATα-inc* lacks a *Hha*I site that is present in *HMLα* (data not shown). In mitotic cells where a galactose-inducible promoter was used to express *HO*, ~30% of the conversions of *MAT***a** used *HMLα* as the donor in

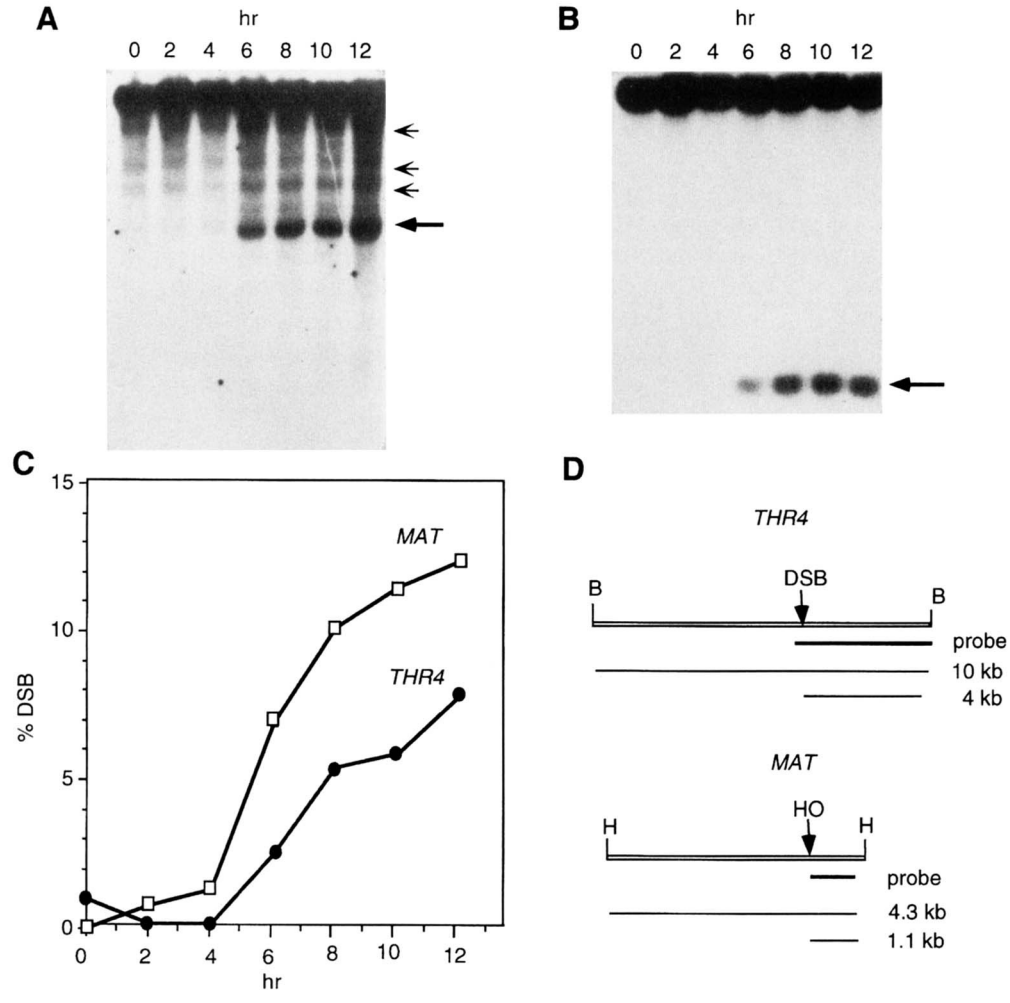

FIGURE 1.—Timing of DSBs formation at *MAT* and *THR4* loci. (A) Timing of appearance of DSB products in a *THR4* proximal region. DNA was extracted from samples taken at various times after induction of meiosis, digested with *Bgl*II, displayed on Southern blots, and probed with fragment indicated on D. The arrow indicates cleavage at the major DSB site and the arrowheads indicate minor DSB sites for *THR4* proximal region that are visible even at 0 hr (WU *et al.* 1993). (B) Timing of appearance of DSB products at *MAT* locus. DNA was digested with *Hin*dIII. Arrow indicates the DSB product. See also comments to A. (C) Graph of the accumulation of DSB product after induction of meiosis. The graph indicates total amount of radioactivity, for each lane, that was in the DSB product bands indicated by arrows in A and B. Only major cut site for the *THR4* proximal region is shown. (D) Structure of *MAT***a** and *THR4* regions. Digestion with *Bgl*II and probing with 4.9-kb *Pst*I fragment of the region immediately upstream of *THR4* (from plasmid pMJ338) illuminates a 10-kb parental restriction fragment and a 4-kb DSB product. Digestion with *Hin*dIII and probing with 650-bp fragment of the region distal to *MAT* locus (an *Xho*I-*Hae*III fragment from plasmid pJH364) illuminates a 4.3-kb parental restriction fragment and a 1.1-kb DSB product. B, *Bgl*II; H, *Hin*dIII.

isogenic diploids (data not shown). This result indicates that the normal donor selection system of *MAT* switching is somehow suppressed in meiosis and the only recombination induced in our system is that occurring between *MAT* loci located on the homologous chromosomes. Under these circumstances, the conversion of *MAT***a** to *MAT*α*-inc* is analogous to any other allelic recombination event.

HO-induced recombination in meiotic cells unable to initiate meiotic DSBs: The utilization of the *SPO13::HO* construct enabled us to analyze recombination events in cells where the normal initiation of meiotic recombination has been prevented by meiotic-deficient mutations. Diploid yeast cells deleted for *RAD50* are incapable of initiating meiotic recombination (MALONE and ESPOSITO 1981; MALONE 1983; BORTS *et al.* 1986). Normally, *rad50* diploids produce inviable spores. *rad50*Δ spores can be rescued in diploids homozygous for *spo13*Δ, which bypasses the first meiotic division. *rad50*Δ *spo13*Δ diploids yield two diploid spores having the same genotype as the initial diploid (MALONE and ESPOSITO 1981). We constructed *rad50*Δ*/rad50*Δ *spo13*Δ*/spo13*Δ *MAT***a***/MAT*α*-inc* diploids (DAM59 and DAM60) heterozygous for a *THR4* marker distal to *MAT* and carrying *lys2::SPO13::HO*. In these strains, the only meiotic recombination that could occur would be that initiated by HO. HO-induced gene conversions of one or both *MAT***a** chromatids to *MAT*α*-inc* occurred frequently (29% of all meioses) resulting in the formation of α-mating Thr^+ diploid spores (Table 3). Thus, *rad50*Δ strains are competent to carry out meiotic gene conversion initiated by DSBs introduced by HO. Analysis of a

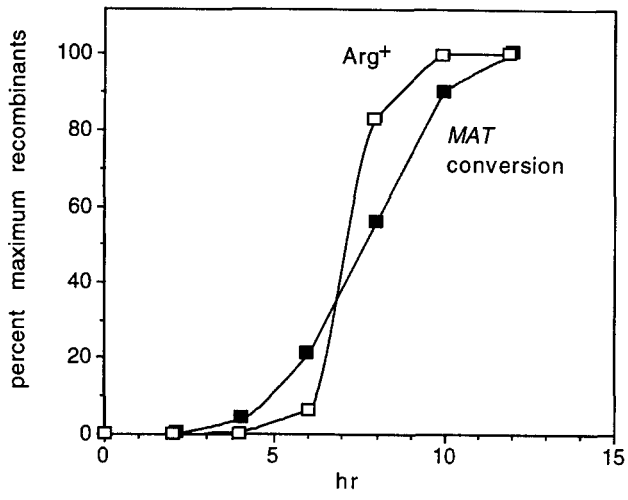

FIGURE 2.—Commitment to the *SPO13::HO*-driven recombination at *MAT* relative to the commitment to meiotic recombination. Commitment to meiotic recombination was measured by scoring Arg^+ prototrophs in return-to-growth experiments with the diploid DAM73 bearing *arg4-BglII/arg4-EcoRV* heteroalleles. Commitment to the *SPO13::HO*-induced recombination was measured by scoring the α-mating cells appearing in the same experiment as a result of recombination between the *MAT***a**/*MATα-inc* heteroalleles. The amount of recombinants is shown as a fraction of the maximum observed level (indicated by the number 100 on the ordinate). The maximum observed values were 8×10^{-3} for the recombination at *ARG4* locus (Arg^+ cells per total cells) and 9×10^{-2} for the recombination at *MAT* (α-mating cells per total cells).

rad50Δ spo13Δ control strain lacking *SPO13::HO* (DAM62) yielded only nonmating Thr^+ spores, indicating that all recombination events described are initiated by *SPO13::HO*.

We induced meiotic DSBs by *SPO13::HO* in another meiotic mutant, *xrs2Δ*. This mutation causes meiotic defects similar to *rad50Δ* (IVANOV *et al.* 1992). Analysis of meiotic products obtained from diploids homozygous for *xrs2Δ spo13Δ* and *lys2::SPO13::HO* (DAM65) also exhibited efficient gene conversion at *MAT* (Table 3). We conclude that *rad50Δ* and *xrs2Δ* strains, normally incapable of initiating meiotic recombination, are competent to carry out meiotic gene conversion initiated by HO to produce α-mating Thr^+ spores.

In both mutant backgrounds dyads containing Thr^- α-mating spores were observed (Table 3). These could arise by one of three different mechanisms: (1) gene conversion at *MAT* associated with crossing over, (2) gene conversion of an ~15 kb chromosomal region including *MAT***a** and *THR4* or (3) loss of the *MAT***a**-*THR4* chromosome as a result of an unrepaired DSB. To distinguish among these possibilities, we inserted additional markers in the *MAT***a**-containing chromosome *III* of the *spo13Δ rad50Δ* mutant. The *URA3* gene was introduced 10 kb proximal to *MAT***a**, and the *ADE1* gene was inserted in place of the *HML* locus of the same chromosome (see MATERIALS AND METHODS). The resultant *lys2::SPO13::HO rad50Δ spo13Δ* diploid

TABLE 2

The *SPO13::HO*-driven recombination at *MAT* locus

Relevant genotype of the diploids	Strain	No. of tetrads[a]					
		2**a**:2α	1**a**:3α	0**a**:4α	3**a**:1α	4**a**:0α	Total
LYS2 MATα-inc / *LYS2 MAT***a**	DL1001, DL1003, DL1015[c]	174	0	0	0	0	174
lys2::SPO13-HO[b] *MATα-inc* / *LYS2 MAT***a**	DAM15	104	6 (5)	20 (15)	0	0	130
lys2::SPO13-HO[b] *MATα-inc* / *LYS2 MAT***a**	DAM75	127	7 (5)	7 (5)	0	0	141
lys2::SPO13-HO[b] *MATα-inc* / *lys2::SPO13-HO MAT***a**	DAM97	54	8 (11)	11 (15)	0	0	73
lys2::SPO13-HO[b] *MATα* / *LYS2 MAT***a**-*inc*	DAM1	152	0	0	18 (9)	27 (14)	197
lys2::SPO13-HO[b] *MATα* / *LYS2 MAT***a**-*inc*	DL1023, DL1042[d]	181	0	0	11 (4)	72 (27)	264
lys2::SPO13-HO[c] *MATα* / *LYS2 MAT***a**-*inc*	DL1024	128	0	0	7 (5)	9 (6)	144

[a] Values in parentheses are percentages.
[b] *SPO13::HO* endonuclease is transcribed in same direction as *LSY2*.
[c] *SPO13::HO* endonuclease is transcribed in opposite direction as *LYS2*.
[d] Data are compiled from several experiments. All strains are isogenic.

TABLE 3

Meiotic recombination induced in meiosis-deficient *spo13* mutants

Phenotype of dyads	No. of dyads		
	rad50Δ / *rad50Δ* (DAM62)	*rad50ΔSPO13::HO* / *rad50ΔSPO13::HO* (DAM59; DAM60)	*xrs2ΔSPO13::HO* / *xrs2ΔSPO13::HO* (DAM65)
2 Nm Thr^+	65	107	74
1 Nm Thr^+:1α Thr^+	0	48	15
2 α Thr^+	0	29	13
1 Nm Thr^+:1α Thr^-	0	33	16
1α Thr^+:1α Thr^-	0	32	10
Others[a]	0	13	8
Total	65	262	136
Diploids of genotype	—o—*MAT***a**———*THR4*— *spo13Δ*		
	—o—*MATα-inc*——*thr4*— *spo13Δ*		

and also homozygous for either *rad50Δ* or *xrs2Δ* mutations were constructed. The *SPO13::HO* gene was introduced into diploids DAM59, DAM60 and DAM65. Because *spo13Δ* diploids fail to undergo the first meiotic division, they produce dyads of two diploid spores. If there is no crossing over between the *MAT* locus and its centromere, and if there is no gene conversion of *MAT***a** to *MATα-inc*, then both spores should be nonmating *MAT***a**/*MATα-inc* (NM). If there is no crossing over between *THR4* and the centromere, both spores of the dyad will be Thr^+.

[a] The exceptional dyads belonging to this class were identified as chromosome loss or *MAT/HMR***a** fusions (see text).

(DAM68) was sporulated, and the resulting pairs of diploid segregants were analyzed. As before, in the absence of HO expression, all spores gave rise to nonmating diploid segregants (data not shown); the appearance of α-mating spores in the progeny of this diploid indicated the HO-induced events. All dyads could be divided into three classes. The majority had no apparent HO-induced events. Approximately 9% of all meioses had one α-mating and one nonmating spore, suggesting that they had experienced one HO-initiated event. An additional 5% apparently had two HO-initiated events, yielding two α-mating spores.

In Figure 3 we present an analysis of the types of segregants in which there was one conversion of *MAT***a** to *MATα-inc*. The dyads in which there were two HO-induced events gave results that were qualitatively similar to those presented in Figure 3 but were more difficult to characterize completely. The various types of dyads were analyzed by genetic and physical assays (see MATERIALS AND METHODS). Because *rad50* diploids have a significant rate of chromosome loss, it was possible to use 5-FOA resistance to select cells that had lost the *URA3*-containing chromosome *III* from Ura^+/Ura^- diploid segregants. Thus, we could determine whether the Ura^- chromosome was linked to *THR4* or *thr4* and could distinguish between the phenotypically identical Classes 1 and 2b (one α-mating Ade^+ Ura^+ Thr^+ segregant and one nonmating Ade^+ Ura^+ Thr^+ segregant) by analyzing the Ura^- derivatives of the α-mating segregant. In Class 1 the Ura^- chromosome was linked to *thr4*, while in the α-mating segregant in Class 2b the Ura^- chromosome was linked to *THR4*. We also could distinguish among phenotypically identical Classes 2a and 3a (one α-mating Ade^+ Ura^+ Thr^- segregant and one nonmating Ade^+ Ura^+ Thr^+ segregant) by analyzing the nonmating segregant. The nonmating segregant in Class 2a was apparently homozygous for *THR4*, while the nonmating partner in Class 3a was heterozygous *THR4/thr4*. Southern blot analysis was used to determine whether the α-mating Thr^- segregants were indeed diploid for the right arm and not partially aneuploid because of the formation of a new telomere at or proximal to *MAT*.

As shown in Figure 3, *SPO13::HO*-induced recombination led to both expected and unexpected types of events. Among these HO-induced *rad50Δ spo13Δ* dyads, 28% of the dyads with one *MAT* conversion contained an α-mating Ade^+ Ura^+ Thr^+ spore (Class 1), as expected from a simple gene conversion event not associated with crossing over. Class 2 (6.8%) contains events where conversion at *MAT* was associated with reciprocal exchange in the interval between *MAT* and *THR4*. These events include two different spore genotypes, depending on segregation of chromosomes during the equational division. These two classes therefore represent both the normally detected and "hidden" cases of gene conversions associated with crossover. Thus 19.6% of the conversions at *MAT* (nine of 46 cases in Classes 1 and 2) were associated with crossing over.

Besides simple gene conversions at *MAT* (with or without crossing over) several other types of events were observed. Dyads belonging to the Classes 3a, 3b and 3c probably arose as a result of conversion involving very long chromosomal regions around *MAT*, including *URA3*, located 10 kb proximal to *MAT* and/or *THR4*, located 15 kb distal to *MAT*. These events represent

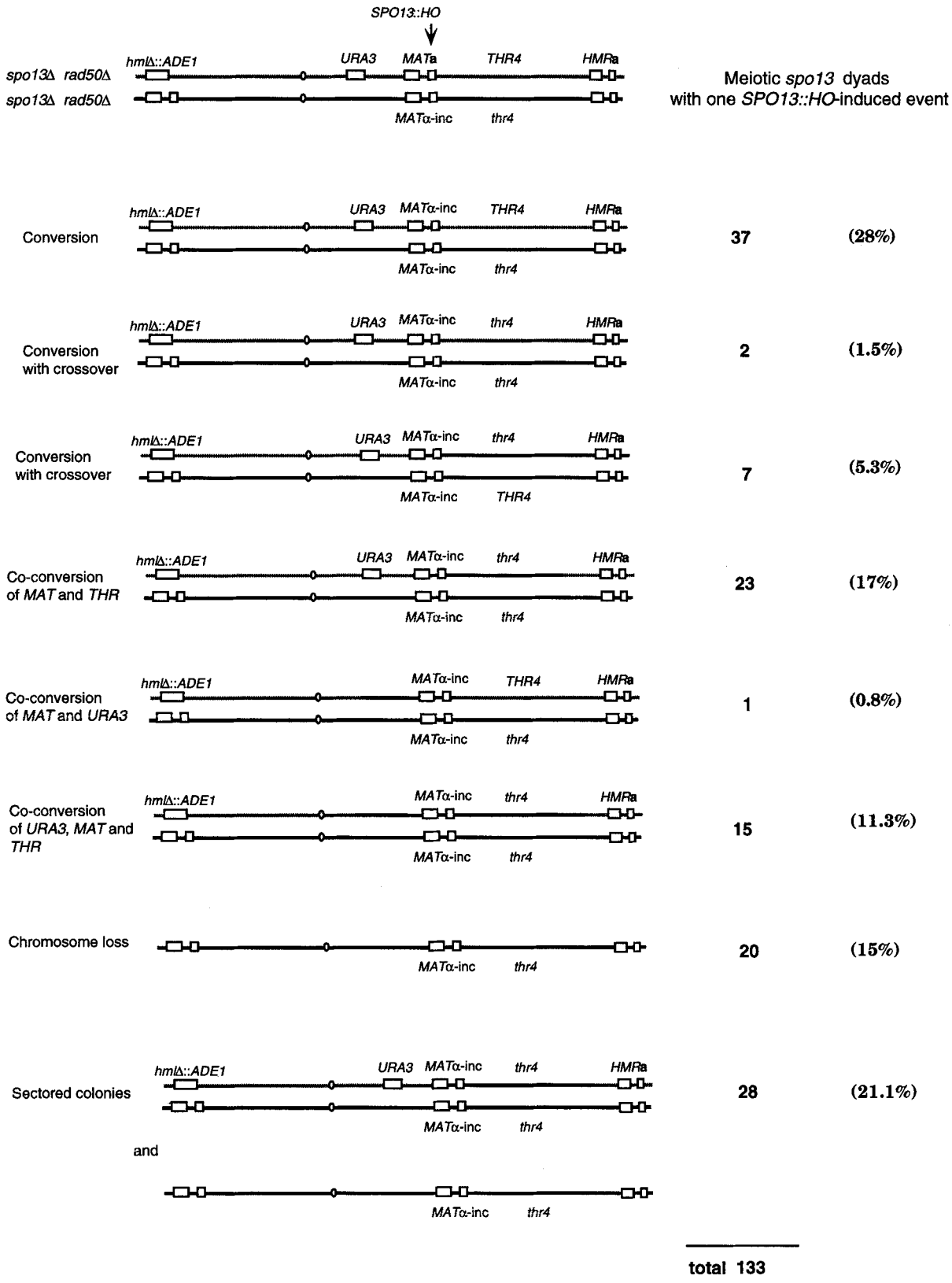

FIGURE 3.—Types of meiotic recombinants induced by *SPO13::HO* in a *rad50Δ spo13Δ* diploid. A *rad50Δ spo13Δ* diploid (DAM68) produces dyads with two diploid spores of parental genotype. When *SPO13::HO* is expressed, gene conversion events of *MAT***a** to *MATα-inc* occur. Meioses in which there had been one HO-induced event are recognized as dyads containing one α-mating (*MATα-inc/MATα-inc*) and one nonmating (*MAT***a**/*MATα-inc*) segregant. The types of recombination events that occurred in the α-mating segregant are shown in this figure, based on the analysis described in the text. The nonmating segregants in these dyads were also analyzed by the chromosome loss and mating tests described in MATERIALS AND METHODS, and Southern blot analysis was carried out when it was necessary to resolve ambiguities between phenotypically identical outcomes.

long gene conversion events, rather than the consequences of a reciprocal exchange event; consequently they exhibit non-Mendelian inheritance of these markers in dyads.

Another class was the 15% of the 1α:1 nonmater dyads that contained an α-mating Ade$^-$ Ura$^-$ Thr$^-$ spore. Cells of Class 4 appear to have lost the *MAT***a**-containing homologue of chromosome *III*. This most likely occurred because of a failure to repair the HO-induced DSB. There were also a significant number of cases where the α-mating segregant was associated with sectoring of *hml::ADE1* or of both *hml::ADE1* and the *MAT*-proximal *URA3* (Class 5). The origin of these Class 5 events cannot easily be explained by a classical meiotic recombination mechanism. One interpretation is that repair was only completed after spore germination, during subsequent mitotic divisions, so that some cells in the colony lost the chromosome (as in Class 4) and others repaired the break (as in Class 1). The fact that an entire, but apparently still broken, chromosome was inherited by some mitotic progeny of the spore suggests that an unrepaired DSB did not prevent the completion of meiosis and the packaging of chromosomes into a spore.

To demonstrate that the unusual events we found in diploid DAM68 are the consequence of deleting *RAD50* and not simply from the creation of HO-induced DSBs, we analyzed meiotic products of an isogenic *RAD50/rad50Δ spo13Δ/spo13Δ lys2::SPO13::HO/lys2::SPO13::HO* diploid (DAM98). Among 102 dyads, 37% contained spores where conversion events at *MAT* occurred (data not shown). Because normal meiotic recombination also occurs, there is frequent crossing over between *MAT* and *THR4* and between *MAT* and *hml::ADE1;* consequently a direct comparison of all classes in Figure 3 is not possible. However, 80% of all events were Class 1 and Class 2 events, compared to 39% in the *rad50* diploid. The remaining events apparently included coconversion of *MAT* and *URA* or coconversion of *MAT* and *URA* associated with conversion of *THR4* or crossing over in the *MAT-THR4* interval. Some of these events could represent cases of a reductional chromosome segregation in *spo13* diploids (Hugerat and Simchen 1993), or they might represent two independent events in these Rad$^+$ cells. No examples of chromosome loss or of sectoring of either *hml::ADE1* or *URA3* were detected. Thus these abnormal repair events could be ascribed to the *rad50* mutation.

The sectored spore colonies of Class 5 (Figure 3) suggested that some *SPO13::HO*-induced events were only completed after spore germination. It was thus formally possible that *all* of the repair events (except for Class 2) actually took place in mitosis, after spores were germinated. To demonstrate directly that some *SPO13::HO*-induced events were completed in meiosis, we examined the timing of recombination by physical analysis of DNA isolated at intervals during meiosis. In these experiments we used diploids DAM95 (*rad50Δ*) and DAM94 (wild type), isogenic to DAM68, but bearing the insertion of *URA3* at the *BUD5* locus (Thierry *et al.* 1990), 3 kb proximal to *MAT* instead of 10 kb as in the DAM68 diploid. This provided a convenient restriction site polymorphism between the two homologues that could be used to determine when *MAT***a** was converted to *MATα-inc*. As shown in Figure 4, in both the *rad50Δ* mutant and in wild-type diploids, the accumulation of conversion product begins at roughly the same time, that is, at 6 hr, which is ~1–2 hr after the appearance of the HO-induced DSBs in these particular experiments (not shown). By the end of the time course the amount of product reached 6.2% for *rad50* mutant and 8% for wild type. These values correspond well to the amount of conversion at *MAT* that was observed by genetic analysis. This result clearly indicates that after DSBs are formed they could be processed into completed recombination products in the *rad50Δ* background. We conclude that in *rad50Δ* mutants the *SPO13::HO*-driven DSBs can be repaired by the classic gene conversion mechanism, but at least some repair events are unusual. Some DSB-initiated events have unexpectedly long conversion tracts, others exhibit chromosome loss and still others appear to have completed recombination in a subsequent mitotic cell division cycle. These results suggest that *RAD50* performs at least one more function in meiotic recombination besides the delivery of DSBs.

SPO13::HO rad50Δ* recombination resembles events in mitotic *rad50Δ GAL::HO* diploids:** In *rad50Δ* mutants the repair of meiotically induced DSBs seems to be partially defective, yielding both expected and unexpected outcomes. We wished to know if these same types of outcomes might be seen in *rad50Δ* mitotic cells in which HO was induced. Previous studies had suggested that the absence of *RAD50* caused a significant delay in the completion of HO-induced mitotic recombination events, at least in part by reducing the rate of 5′ to 3′ degradation of the DSB ends (Sugawara and Haber 1992). However, previous assays would not have detected some of the types of outcomes shown in Figure 3. We therefore introduced into strain DAM68 a *TRP1*-containing centromeric plasmid pFH800 carrying the HO gene under the control of a galactose-inducible promoter (Nickoloff *et al.* 1986). After *GAL::HO* induction in these mitotic cells, where *SPO13::HO* is not expressed, ~50% of the originally nonmating (*MATa**/*MATα-inc*) diploids had become α-mating. The types of events we recovered are shown in Figure 5. While not all classes can be unequivocally identified, it is clear that the spectrum of events is surprisingly similar to what we observed in meiosis (Figure 3). Gene conversions associated with very long conversion tracts (10–30 kb) were found in >15% of the cases, while chromosome loss accounted for 3.5%. In the wild-type control (DAM98 transformed by pFH800 plasmid) there were no cases of long conversion tracts (coconversion of *MAT* and *URA* or coconversion of *MAT, URA* and *THR*). We conclude that one of the functions *RAD50* plays in

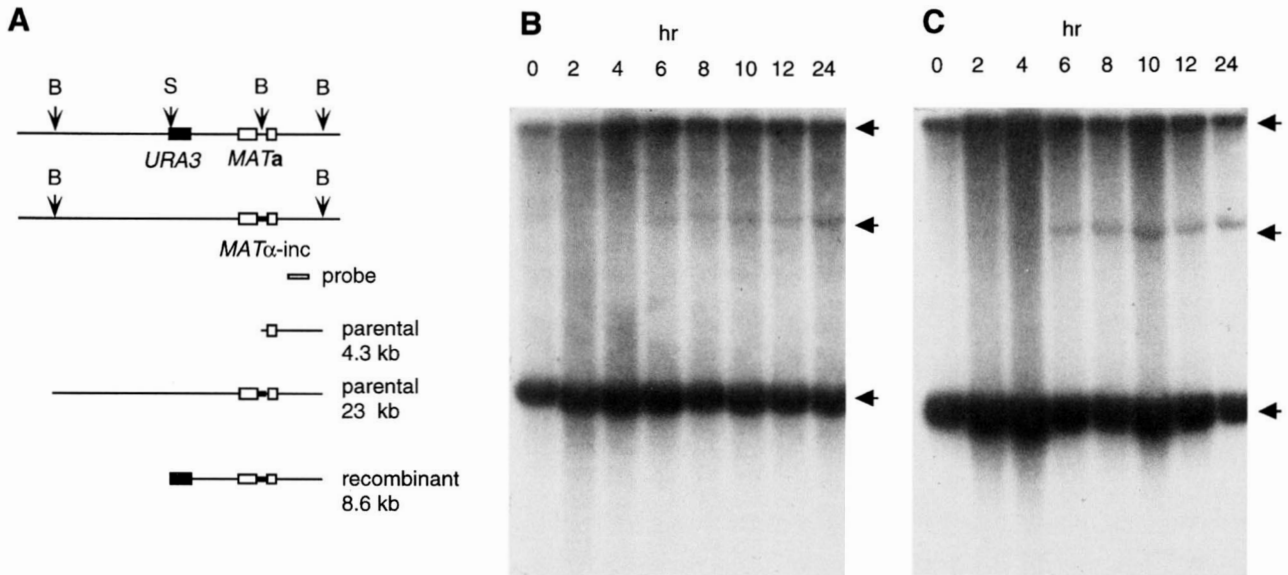

FIGURE 4.—Timing of the *SPO13::HO*-induced recombination at *MAT*. (A) Structure of the *MAT* region in DAM94 and DAM95 diploids. Digestion with *Bgl*II (B) and *Sma*I (S) and probing with 650-bp *Xho*I-*Hae*III fragment of the region distal to the *MAT* locus illuminates restriction fragments from parents: 23 kb (*MATα-inc* parent) and 4.3 kb (*MAT***a** parent). Gene conversion at the *MAT***a** locus yields a *Sma*I-*Bgl*II fragment of 8.6 kb. (B) Timing of appearance of conversion product at the *MAT***a** locus in DAM95 diploid homozygous for the *rad50*Δ mutation. DNA was extracted from samples taken at various times after induction of meiosis, digested with *Sma*I and *Bgl*II, displayed on Southern blots, and probed with fragment indicated in A. Positions of fragments diagnostic of parental chromosomes and recombinant product are indicated. (C) Timing of appearance of conversion product at *MAT* locus in the wild-type diploid DAM94.

meiotic recombination is the same one it plays in mitotic recombination, but it was impossible to identify it until meiotic DSBs were provided in an artificial manner in a *rad50*Δ strain. In both mitosis and meiosis DSBs can be repaired successfully by gene conversion in the absence of *RAD50*, but there are changes in the types of outcomes that are observed, suggesting that *RAD50* participates in later steps of recombination, after the induction of DSBs.

In both meiotic and mitotic experiments we also found a class of events in which there had been a fusion between *MAT* and *HMR***a**. These events had nonmating Ade^+ Ura^+ Thr^- phenotype and were identified by their mating behavior (see MATERIALS AND METHODS) and confirmed by Southern blots, probed with DNA distal to *MAT* (HABER *et al.* 1980; KLAR and STRATHERN 1984) (data not shown). In mitosis these events were observed in 6% of the colonies derived from wild-type *GAL::HO*-induced cells and in 9% of the cases in *rad50*Δ mutants. In meiosis these events were observed in *rad50*Δ mutants in 10% of the cases where *SPO13::HO* was induced. These events were eliminated in the isogenic diploid DAM77 in which the *HMR* locus of the *MAT***a** chromosome was deleted and replaced by the *LEU2* gene (data not shown), while all other classes of events were similar to those shown in Figure 3 (data not shown).

DISCUSSION

A *SPO13::HO*-induced DSB, appearing at the time that normal meiotic DSBs appear, initiates recombination events at approximately the same time and with the same kinetics as normal meiotic DSBs. This enables us to compare directly the properties of HO-induced recombination in both meiotic and mitotic cells. An important difference between this study and a previous one using a galactose-induced HO gene (KOLODKIN *et al.* 1986) is that we recovered tetrads with 3α:1**a** segregants, as well as 4α:0**a** segregants that we and they observed. Thus we could be sure that at least the 3:1 events were initiated after meiotic DNA replication. The proportion of meioses with only one *SPO13::HO*-initiated event could be underestimated. As suggested by KOLODKIN *et al.* (1986), it is possible that the HO-cut *MAT***a** is repaired by its sister, uncut *MAT***a** allele rather than *MATα-inc*, so that the tetrad would be restored to 2 *MAT***a**:2 *MATα-inc*. However, it is evident from the data in Table 2 that there is not highly preferential repair of the DSB from the sister chromatid, as there are many 3α:1**a** events.

The use of the *SPO13::HO* gene enables us to examine recombination events in *rad50* or *xrs2* diploids that are otherwise devoid of recombination. This permits us to ask if *RAD50* and *XRS2* play roles in meiotic recombination after the creation of a DSB. We show that not all of the HO-induced DSBs in a *rad50* diploid are repaired with the kinetics expected for normal meiosis; some DSBs apparently persist even through chromosome segregation, spore formation and germination and are only repaired in subsequent mitotic cell divisions. We draw this conclusion from the recovery of sectored colonies derived from germinated spores, where one half of the sector had lost the chromosome that HO apparently

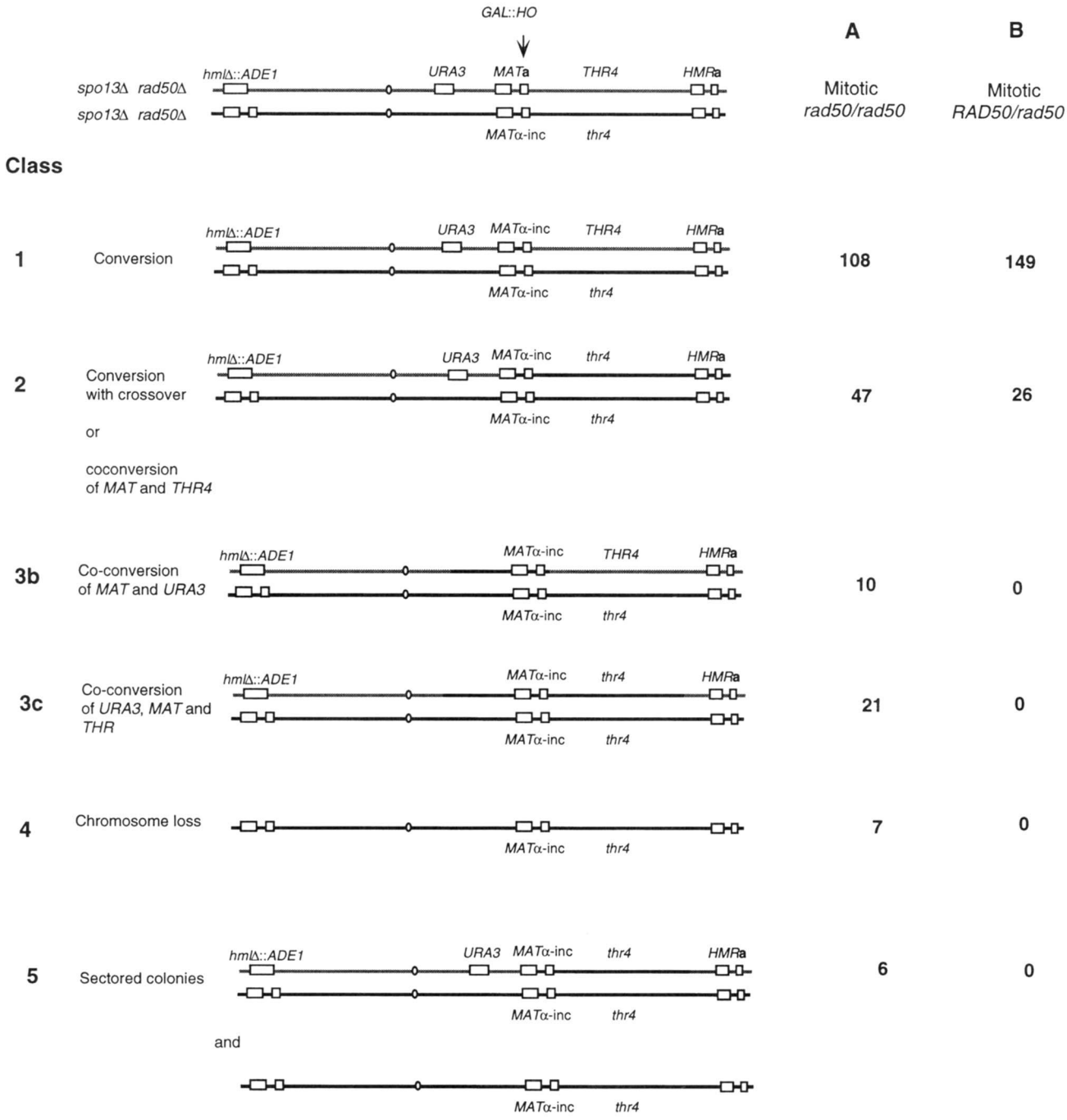

FIGURE 5.—HO-induced conversion of *MAT***a** to *MATα-inc* in mitotic cells. A galactose-induced *HO* gene was used to induce conversion at the *MAT* locus. As described in the text, 96% of these events arose by conversion of *MAT***a** to *MATα-inc,* with the remaining events using *HMLα* as the donor. The types of diploids obtained in *rad50*Δ and wild-type cells are shown.

cleaved, while the other half of the sector had retained and repaired this chromosome by recombination. We believe it is unlikely that these events can be explained by the unscheduled expression of *SPO13::HO* in the germinating mitotic cells, though we cannot completely rule out this possibility. First, we did not observe these events analyzing meiotic products of *RAD50* strains bearing the same *SPO13::HO* construct. Second, we found no evidence for vegetative expression of *SPO13::HO* by screening for the formation of α-mating (*MATα-inc*/*MATα-inc*) diploids from *MAT***a**/*MATα-inc* vegetative cells. Instead, we suggest that an unrepaired DSB does not prevent cells from undergoing the mitotic-like chromosome segregation of *spo13* diploids. This suggests that *RAD50* continues to play an important part in meiotic recombination after its role in creating DSBs. In mitotic cells, *rad50* mutant diploids exhibit an altered spectrum of recombination events compared to the wild type. Thus, even in mitotic cells, the absence of *RAD50* does more than simply delay the kinetics of completing HO-induced recombination (SUGAWARA and HABER 1992; IVANOV *et al.* 1994).

It is noteworthy that spore formation was completed

in cells that apparently contained an unrepaired DSB. Such behavior would occur only if there is no strong meiosis I or meiosis II checkpoint that operates to delay spore formation until broken chromosomes are repaired. This suggestion does not preclude the idea that the process of germination could have a DNA damage-sensitive checkpoint.

Similarities and differences between HO-induced and meiotic DSBs: The results we present suggest that, in a general way, an HO-induced DSB can initiate recombination in meiotic cells. The timing of recombination appears to be quite similar between events at *MAT* and those at the *THR4* locus (for DSBs) and at the *ARG4* locus (for commitment to recombination). The time between the appearance of DSBs and subsequent recombined recombination products is surprisingly slow for HO-induced breaks (both in mitosis and meiosis) and for meiotic DSBs; in all cases, there is about a 1-hr delay (CAO *et al.* 1990; WHITE and HABER 1990). However, there are some apparent differences between the two types of breaks. First, several studies have suggested that meiotic DSBs are regulated, so that the same region is rarely cut on both sister chromatids; consequently the frequency of aberrant 6:2 or 8:0 tetrads is lower than predicted, based on the frequencies of single events and from aberrant 4:4 tetrads (PORTER *et al.* 1993; ALANI *et al.* 1994). This does not seem to be the case with *SPO13::HO,* as there were many instances where both *MAT***a** targets were cut and converted. Second, there may be differences in the processing of the two types of DSBs. HO generates 3′ overhanging ends while meiotic DSBs have blunt or 5′ overhanging ends. This might greatly influence how they are subsequently processed. For example, although a *rad50* deletion mutation slows down 5′ to 3′ exonuclease degradation of an HO-induced DSB in mitotic cells, a *rad50S* mutation has no greater effect and permits exonucleolytic processing in mitotic cells (E. IVANOV and J. E. HABER, unpublished observations). We have not yet determined if *rad50S* prevents the processing of *SPO13::HO*-induced DSBs. The fact that HO-induced DSBs do not disappear by 12 hr in meiosis (Figure 1) does not necessarily mean that these breaks are not processed in *rad50S* background. This could be because the *SPO13::HO* fusion is expressed for a long time in meiosis, or that HO activity turns over less rapidly than in mitotic cells, or because the culture is not very synchronous.

An alteration in mating-type donor preference in meiosis: One surprising outcome of these studies is the discovery that there is a distinctive difference in the frequency with which the silent mating type donors *HML* and *HMR* are used to repair a DSB at *MAT* in meiotic and mitotic cells. In mitotic *MAT***a**/*MAT*α-*inc* cells, *MAT***a** is repaired by *HML*α ~30% of the time, with the remaining conversions coming from *MAT*α-*inc* (HABER *et al.* 1980; KLAR and STRATHERN 1984; A. MALKOVA and J. E. HABER, unpublished observations). In meiotic cells *HML*α was not used as a donor once in 36 cases of DSB repair at *MAT*. This constraint does not reflect a general inhibition of ectopic interactions between homologous DNA sequences, as we have shown previously that a *leu2* allele inserted adjacent to *HML* could recombine with a *leu2* allele either at the *LEU2* locus or adjacent to *MAT,* at frequencies that were not substantially different from other ectopic and allelic interactions (LICHTEN *et al.* 1987). Instead there may be a change in the degree of accessibility of the silent sequences, located near the telomeres of chromosome *III,* that is meiotic-specific. Further experiments are underway to explore this phenomenon.

What is the role of *RAD50* in meiosis and mitosis: We propose that *RAD50* is part of (or necessary for the formation of) a structure in mitosis and meiosis that connects sister chromatids and regulates their recombinational behavior. This notion is based on several observations. First, in mitotic cells, *RAD50* appears to be essential for the ability of sister chromatids to recombine, or protect each other, during the G2 phase of the cell cycle after X-irradiation (FABRE *et al.* 1984). Second, the absence of this structure could result in the persistence of DNA damage (unrepaired by a sister chromatid) that would then result in the hyper-recombinational phenotype of *rad50*Δ strains. Third, the nonhomologous repair of a DSB in the S/G2 phase of the cell cycle by end-filling of misaligned DNA ends is prevented by a deletion of *RAD50,* while the deletion-repair of these DSBs that predominates in G1 cells is much less affected by *RAD50* (MOORE and HABER 1996).

We further suggest that Rad50p is associated with the axial elements that are essential for synaptonemal complex (SC) formation. We imagine that *RAD50,* in the context of the axial element, is part of the complex that then creates meiotic DSBs. This implies that the cleavage of DNA by a meiotic nuclease does not occur simply at any DNaseI hypersensitive site but only at those sites that lie within the axial element (or its precursor) that forms between sister chromatids and therefore come into contact with the meiotic endonuclease. Only a fraction of total DNA appears to be involved in the formation of axial elements and subsequently in SC (ROEDER 1995). Whether some sequences are preferentially included in these structures (and therefore would be more frequently be involved in recombination) is not known, but this might account for the observation that some relatively weakly transcribed genes such as *HIS4* and *LEU2* are nevertheless very prominent hotspots and why an 8-kb "cassette", when inserted at five different chromosomal locations, exhibits a 20-fold range in allelic recombination rates (LICHTEN *et al.* 1987). Thus, the degree to which a particular region is likely to be active in recombination will depend not only on the intrinsic property of the sequence but also its probability of being included in the structure where DSBs are generated.

RAD50 affects the way in which an HO-induced DSB is repaired. Our previous studies of *MAT* switching had

suggested that the absence of *RAD50* decreased the rate of 5′ to 3′ exonuclease degradation and also delayed later unidentified steps in the completion of recombination. However, a *rad50* deletion did not reduce the apparent efficiency of DNA repair or cell viability (IVANOV *et al.* 1994). In the present study, we have examined the role of *RAD50* in a diploid where the failure to repair the DSB is not lethal; the broken chromosome simply gets degraded and lost. In this case, the absence of *RAD50* quite dramatically changes the outcomes, both in mitosis and in meiosis. First, a significant number of cells lose the broken chromosome, indicating that they failed to recombine properly. Second, there were many long coconversion events around *MAT* that were not seen in wild-type cells. Given that *rad50* cells appear to have less rapid formation of 3′ ended single-stranded DNA tails, it is unclear why coconversion should appear to be greater. One possibility is that interhomologue as well as sister chromatid interactions are deranged, so that it is difficult for interacting partners for recombination to identify each other (*i.e., RAD50* plays a direct role in the search for homology).

In summary, we conclude *RAD50* is not essential for the completion of recombination induced by a DSB in meiotic cells. However this system has allowed us to determine that the roles that *RAD50* plays in homologous recombination are much more complex than were previously appreciated.

The system we have developed allows us to compare directly the way in which a known DSB promotes recombination in mitotic and meiotic cells. Several important issues need to be addressed. If an HO cut site is inserted in non-*MAT* sequences, will the proportion of gene conversion associated with crossing over be different in mitosis and meiosis? Will HO-induced DSBs lead to the assembly of similar recombination complexes as have recently been visualized for normal meiotic DSBs (BISHOP 1994)? Will an HO-induced crossover between a chromosome pair produce a functional chiasmata capable of directing their segregation? Through the use of *SPO13::HO,* it is now possible to address these and other questions about the ways meiotic recombination differs from mitotic recombination.

We thank N. KLECKNER, M. LICHTEN, C. FAIRHEAD, N. SCHULTES and X. WU for providing plasmids. We also thank members of the HABER lab, especially E. IVANOV, G. BOSCO and A. HOLMES for their kindness in probing Southern blots while A.M. was expecting her daughter. This work was supported by National Science Foundation grant MCB 9424330 SGER and National Institutes of Health (NIH) grant GM-29376 awarded to J.E.H. and NIH grant GM-40452 to D.D. M.H. was supported by a Lucille P. Markey Scholar's Award in Biomedical Sciences.

LITERATURE CITED

ALANI, E., S. SUBBIAH and N. KLECKNER, 1989 The yeast *RAD50* gene encodes a predicted 153-kd protein containing a purine nucleotide binding domain and two large heptad-repeat regions. Genetics **122:** 47–57.

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.

ALANI, E., R. A. REENAN and R.D. KOLODNER, 1994 Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae.* Genetics **137:** 19–39.

BISHOP, D. K., 1994 RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell **79:** 1081–1092.

BOEKE, J. D., F. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–346.

BORTS, R. H., M. LICHTEN and J. E. HABER, 1986 Analysis of meiosis-defective mutations in yeast by physical monitoring of recombination. Genetics **113:** 551–567.

BUCKINGHAM, L. E., H.-T. WANG, R. M. ELDER, R. M. MCCARROL, M. R. SLATER *et al.,* 1990 Nucleotide sequence and promoter analysis of *SPO13,* a meiosis-specific gene of *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **87:** 9406–9410.

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.

CHATTOO, B. B., F. SHERMAN, D. A. ATUBALIS, T. A. FIELLSTEDT, D. MEHNERT *et al.,* 1979 Selection of *lys2* mutants on the yeast *Saccharomyces cerevisiae* by the utilization of α-aminoadipate. Genetics **93:** 51–65.

COLLEAUX, L., L. D'AURIOL, F. GALIBERT and B. DUJON, 1988 Recognition and cleavage of the intron-encoded omega transposase. Proc. Natl. Acad. Sci. USA **85:** 6022–6066.

DE MASSY, B., and A. NICOLAS, 1993 The control *in cis* of the position and the amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae.* EMBO J. **12:** 1459–1466.

DE MASSY, B., V. ROCCO and A. NICOLAS, 1995 The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae.* EMBO J. **14:** 4589–4598.

EIBEL, H., and P. PHILIPPSEN, 1883 Identification of the cloned *Saccharomyces cerevisiae LYS2* gene by an integrative transformation approach. Mol. Gen. Genet. **91:** 66–73.

FABRE, F., A. BOULET and H. ROMAN, 1984 Gene conversion at different points in the mitotic cycle of *Saccharomyces cerevisiae.* Mol. Gen. Genet. **195:** 139–143.

GOYON, C., and M. LICHTEN, 1993 Timing of molecular events in meiosis in *Saccharomyces cerevisiae:* stable heteroduplex DNA is formed late in meiotic prophase. Mol. Cell. Biol. **13:** 373–382.

HABER, J. E., 1995 *In vivo* biochemistry: physical monitoring of recombination induced by site-specific endonucleases. BioEssays **17:** 609–620.

HABER, J. E., and M. HEARN, 1985 *RAD52*-independent mitotic gene conversion in *Saccharomyces cerevisiae* frequently results in chromosome loss. Genetics **111:** 7–22.

HABER, J. E., D. MASCIOLI and D. T. ROGERS, 1980 Illegal transposition of mating type genes in yeast. Cell **20:** 519–528.

HAWTHORNE, D. C., 1963 A deletion in yeast and its bearing on the structure of the mating type locus. Genetics **77:** 639–650.

HUGERAT, Y., and G. SIMCHEN, 1993 Mixed segregation and recombination of chromosomes and YACs during single-division meiosis in *spo13* strains of *Saccharomyces cerevisiae.* Genetics **135:** 297–308.

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.

IVANOV, E. L., N. SUGAWARA, C. I. WHITE, F. FABRE and J. E. HABER, 1994 Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **14:** 3414–3425.

KAISER, C., S. MICHAELIS and A. MITCHELL, 1994 *Methods in Yeast Genetics.* Cold Spring Harbor Press, Cold Spring Harbor, NY.

KEENEY, S., and N. KLECKNER, 1995 Covalent protein-DNA complexes at the 5′ termini of meiosis-specific double strand breaks in yeast. Proc. Natl. Acad. Sci. USA **92:** 11274–11278.

KLAPHOLZ, S., C. S. WADDELL and R. E. ESPOSITO, 1985 The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:** 187–216.

KLAR, A. J. S., and J. N. STRATHERN, 1984 Resolution of recombination intermediates generated during yeast mating type switching. Nature **310:** 744–748.

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.

KOSTRIKEN, R., J. N. STRATHERN, A. J. S. KLAR, J. B. HICKS and F. HEFFRON, 1983 A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. Cell **35:** 167-174.

KRAMER, K. M., and J. E. HABER, 1993 New telomeres in yeast are initiated with a highly selected subset of $TG_{1\text{-}3}$ repeats. Genes Dev. **7:** 2345–2356.

LICHTEN, M., R. H. BORTS and J. E. HABER, 1987 Meiotic gene conversion and crossing-over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics **115:** 233–246.

LIU, J., T. C. WU and M. LICHTEN, 1995 The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA-protein intermediate. EMBO J. **14:** 4599–4608.

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

MALONE, R. E., and R. E. ESPOSITO, 1981 Recombinationless meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1:** 891–901.

MALONE, R. E., T. WARD, S. LIN and J. WARING, 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. BULLARD, M. HERMISTON, R. RIEGER, M. COOL *et al.*, 1991 Isolation of mutants defective in early steps of meiotic recombination in the yeast *Saccharomyces cerevisiae*. Genetics **128:** 79–88.

MOORE, J. K., and J. E. HABER, 1996 Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. Mol. Cell. Biol. (in press).

NICKOLOFF, J. A., E. Y. CHEN and F. HEFFRON, 1986 A 24-base-pair DNA sequence from the *MAT* locus stimulates intergenic recombination in yeast. Proc. Natl. Acad. Sci. USA **83:** 7831–7835.

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 An initiation site for gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–39.

PORTER, S., M. A. WHITE and T. D. PETES, 1993 Analysis of meiotic recombination events near a recombination hotspot in the yeast *Saccharomyces cerevisiae*. Genetics **134:** 5–19.

RESNICK, M. A., and P. MARTIN, 1976 The repair of double-strand breaks in nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. **143:** 119–129.

ROEDER, G. S., 1995 Sex and the single cell: meiosis in yeast. Proc. Natl. Acad. Sci. USA **92:** 10450–10456.

ROTHSTEIN, R., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SCHERTHAN, H., J. LOIDL, T. SCHUSTER and D. SCHWEIZER, 1992 Meiotic chromosome condensation and pairing in *Saccharomyces cerevisiae* studied by chromosome painting. Chromosoma **101:** 590–595.

SHERMAN, F., and H. ROMAN, 1963 Evidence for two types of allelic recombination in yeast. Genetics **48:** 255–261.

SUGAWARA, N., and J. E. HABER, 1992 Characterization of double-strand break induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. **12:** 563–575.

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

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

THIERRY, A., C. FAIRHEAD and B. DUJON, 1990 The complete sequence of the 8.2 kb segment left of *MAT* on chromosome *III* reveals five ORFs, including a gene for a yeast ribokinase. Yeast **6:** 521–534.

THORNE, L. W., and B. BYERS, 1993 Stage-specific effects of X-irradiation on yeast meiosis. Genetics **134:** 29–42.

WANG, H. T., S. FRACKMAN, J. KOWALISYN, R. E. ESPOSITO and R. ELDER, 1987 Developmental regulation of *SPO13*, a gene required for separation of homologous chromosomes at meiosis I. Mol. Cell. Biol. **7:** 1425–1435.

WEIFFENBACH, B., D. T. ROGERS, J. E. HABER, M. ZOLLER, D. W. RUSSEL *et al.*, 1983 Deletion 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.

WHITE, C. I., and J. E. HABER, 1990 Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. **9:** 663–673.

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

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

ZENVIRTH, D., T. ARBEL, A. SHERMAN, M. GOLDWAY, S. KLEIN *et al.*, 1992 Multiple sites for double-strand breaks in whole meiotic chromosomes of *Saccharomyces cerevisiae*. EMBO J. **11:** 3441–3447.

Communicating editor: S. JINKS-ROBERTSON