MOLECULAR AND CELLULAR BIOLOGY, Jan. 1993, p. 373–382
0270-7306/93/010373-10$02.00/0

Vol. 13, No. 1

Timing of Molecular Events in Meiosis in *Saccharomyces cerevisiae*: Stable Heteroduplex DNA Is Formed Late in Meiotic Prophase

CHRISTOPHE GOYON† AND MICHAEL LICHTEN*

Laboratory of Biochemistry, National Cancer Institute, Building 37, Room 4D14, National Institutes of Health, Bethesda, Maryland 20892

Received 19 June 1992/Returned for modification 11 August 1992/Accepted 8 October 1992

To better understand the means by which chromosomes pair and recombine during meiosis, we have determined the time of appearance of heteroduplex DNA relative to the times of appearance of double-strand DNA breaks and of mature recombined molecules. Site-specific double-strand breaks appeared early in meiosis and were formed and repaired with a timing consistent with a role for breaks as initiators of recombination. Heteroduplex-containing molecules appeared about 1 h after double-strand breaks and were followed shortly by crossover products and the first meiotic nuclear division. We conclude that parental chromosomes are stably joined in heteroduplex-containing structures late in meiotic prophase and that these structures are rapidly resolved to yield mature crossover products. If the chromosome pairing and synapsis observed earlier in meiotic prophase is mediated by formation of biparental DNA structures, these structures most likely either contain regions of non-Watson-Crick base pairs or contain regions of heteroduplex DNA that either are very short or dissociate during DNA purification. Two loci were examined in this study: the normal *ARG4* locus, and an artificial locus consisting of an *arg4*-containing plasmid inserted at *MAT*. Remarkably, sequences in the *ARG4* promoter that suffered double-strand cleavage at the normal *ARG4* locus were not cut at significant levels when present at *MAT*::*arg4*. These results indicate that the formation of double-strand breaks during meiosis does not simply involve the specific recognition and cleavage of a short nucleotide sequence.

Meiosis is characterized in most eucaryotes by the pairing of homologous chromosomes and by high levels of genetic recombination. It has been suggested that an early step in chromosome pairing is the joining of parental strands in hydrogen-bonded hybrid structures that are intermediates in recombination (9, 18, 32, 42). Genetic studies of fungal meiosis indicate that hybrid DNA is formed during meiotic recombination (for a review, see reference 14). Largely on the basis of these studies, several molecular models of meiotic recombination have been proposed (15). Although these differ in detail, they all involve (i) initiation by breakage of one or more DNA strands and the generation of single-strand DNA, (ii) invasion of a duplex DNA molecule by single-strand DNA from another chromosome to form a paired structure that contains hybrid DNA, and (iii) the resolution of this intermediate to regenerate duplex DNA molecules that contain heteroduplex DNA, a form of hybrid DNA in which parental strand contributions are joined by Watson-Crick base pairing.

Recent studies indicate that double-strand DNA breaks play a role in initiating meiotic recombination in *Saccharomyces cerevisiae*. Transient double-strand breaks occur during meiosis in sequences associated with high levels of meiotic recombination (8, 43). These breaks appear early in meiotic prophase, just before or at the same time that cells commit to meiotic levels of recombination and that structures associated with chromosome pairing first appear (12, 28, 43). The breaks are processed to form molecules that contain free 3′ single-strand overhangs (44). Joint molecules formed by using these molecules as substrates would have a strand composition and polarity consistent with that observed in the final products of meiosis (21, 23). Mutations that block either the formation or the processing of these breaks also confer defects in meiotic recombination and chromosome pairing (1, 8, 18). These observations, coupled with numerous observations that artificially induced breaks efficiently promote recombination in both mitotic (10, 25, 27, 29, 35, 36) and in premeiotic cells (19), have led to the suggestion that meiotic recombination in yeasts is initiated by double-strand DNA breaks.

Although double-strand breaks appear early in meiotic prophase, fully duplex recombined DNA molecules do not appear until late in meiotic prophase, just before the first meiotic nuclear division (12, 28). A similar gap in time has been observed in studies of double-strand break-induced mitotic recombination (10, 29, 36, 47). It is presumably during this period that recombination intermediates form and chromosomes pair. Little is known about the structure of these intermediates, although DNA molecules with the appearance of recombination intermediates have been isolated from meiotic yeast cells (4). It has been assumed that these intermediates contain heteroduplex DNA. Testing this suggestion requires the ability to detect heteroduplex DNA molecules. Such a technique has recently been developed and used to directly detect heteroduplex molecules in the spore products of meiosis (21).

We have used this technique to examine DNA extracted from synchronous meiotic cultures of *S. cerevisiae*. We determined, at two loci, the time of appearance of heteroduplex DNA relative to the times of appearance of double-strand breaks and of mature recombined molecules. Double-strand breaks occurred early in meiosis at both loci, and the timing of formation and repair of these breaks was consistent

* Corresponding author. Electronic mail address: lichten%bchem.dnet@dxi.nih.gov.

† Present address: Institut de Génétique et Microbiologie, Bâtiment 400, Université Paris-Sud, 91405 Orsay Cedex, France.

TABLE 1.

Strain	Genotype[a]
MJL1059	*MATa arg4-nsp* *MATα arg4-bgl*
MJL1102	*MATa DED82-URA3-DED81 arg4-nsp* *MATα DED82---------DED81 arg4-bgl*
MJL1068	*MATa::URA3–arg4-nsp leu2-R arg4-nsp bgl* *MATα::URA3–arg4-bgl leu2-K arg4-nsp bgl*
MJL1124	*MATa::URA3–arg4-nsp LEU2 arg4Δ (Hpa1-Hpa1)::URA3* *MATα::URA3–arg4-bgl leu2-K arg4Δ (Hpa1-Hpa1)::URA3*

[a] In addition to the markers listed in this table, all strains are homozygous for the following markers: *ura3 lys2 ho::LYS2*.

with a role as initiators of meiotic recombination. Both heteroduplex DNA and crossover products appeared shortly before the first meiotic nuclear division, with a considerable period separating the formation of double-strand breaks from the appearance of stable heteroduplex molecules. These findings are consistent with the suggestions that stable intermediates containing heteroduplex DNA are formed only late in meiosis and that such structures are rapidly resolved.

We also observed a striking context effect on the susceptibility of a site to double-strand cleavage during meiosis. Two loci were used in this study: the normal *ARG4* locus, and an artificial locus consisting of an *arg4*-containing plasmid inserted at the *MAT* locus. A site in the *ARG4* promoter region that suffered cleavage when at the normal *ARG4* locus (43) was not cut when present in the *arg4* insert at *MAT*. We conclude that DNA sequence alone does not determine whether or not a site will be cut during meiosis. Other factors must play a predominant role in determining which sites are recognized and broken.

MATERIALS AND METHODS

Yeast strains. All yeast strains used were isogenic derivatives of SK1 (17). Relevant genotypes are listed in Table 1. *leu2-R* and *leu2-K* have been described (20). *arg4-nsp* is a G · C to C · G mutation in the initiator ATG codon of *ARG4*; *arg4-bgl* is a 4-bp fill-in that destroys a *Bgl*II site at +1272 bp relative to the *ARG4* translation start (26). MJL1102 is homozygous for a C · G to T · A change, constructed in vitro (46), that removes a *Nsp*HI site at +977 bp without changing the *ARG4* amino acid sequence. MJL1102 is also homozygous for two mutations, one at −1746 bp that destroys a *Bam*HI site and one at −2305 bp that creates an *Eco*RV site (39). The *DED82-URA3-DED81 arg4-nsp* chromosome contains a 1.2-kb *URA3 Hin*dIII (33) fragment inserted at this *Eco*RV site. A schematic representation of the *ARG4* region in MJL1102 is shown in Fig. 1A.

Genetic markers in the *ARG4* region were introduced by the following procedure, to ensure sequence identity with cloned *ARG4* sequences (3) between two *Hpa*I sites at −317 and +1743 bp. Sequences between the two *Hpa*I sites were replaced by the 1.2-kb *Hin*dIII *URA3* fragment by one-step disruption (34) to form *arg4Δ (Hpa1-Hpa1)::URA3*. These strains were transformed back to *ARG4* with a 3.3-kb *Pst*I fragment containing *ARG4* (3). *arg4-nsp*, *arg4-bgl*, and markers upstream of *ARG4* were then introduced by two-step replacement (38) with 5-fluoro-orotic acid to select for plasmid loss (6), by transformation with a DNA fragment (from −2795 to +1880 bp) that contained the desired point mutations and a copy of *URA3* between *DED81* and *DED82*, or by transformation of *arg4Δ::URA3* strains with a DNA fragment (−2795 to +1880 bp) containing *ARG4* and linked silent mutations. *arg4-nsp bgl* strains were derived from meiotic segregants of MJL1059. All genotypes were confirmed by Southern blot analysis. These strains are identical in sequence to cloned *ARG4* sequences from −317 to +1743 bp; it is likely (but not certain) that in MJL1102 the region upstream of *ARG4* from −2305 to −317 bp is also identical to cloned sequences.

MJL1068 and MJL1124 contain nontandem duplications of the *MAT* locus separated by a pBR322-based insert (20). The plasmids used to create these inserts were constructed as follows. A 3.5-kb *Eco*RI-*Hin*dIII fragment containing the *MATa* locus (2) was inserted between the *Eco*RI and *Hin*dIII sites of pBR322, and the *URA3* fragment was inserted at the *Hin*dIII site. The 5′ overhanging ends of the pBR322 *Sal*I site were converted to blunt ends with T4 DNA polymerase and ligated to *Bam*HI linkers. The 3′ overhanging ends of the relevant *arg4 Pst*I fragment were removed with T4 DNA polymerase, *Bam*HI linkers were added, and the *arg4 Pst*I fragments were then inserted into the modified *MAT-URA3*-pBR322 plasmid. Methods used were as previously described (37).

Genetic analysis of tetrads from MJL1102 and random spores from MJL1068. The *arg4* allele composition of spore colonies from tetrads of MJL1102 was determined as follows. Spore colonies were patched onto YPD plates, cross-stamped with *arg4-nsp* and *arg4-bgl* tester strains, incubated overnight at 30°C, and replica plated to sporulation plates (40). After 2 days of incubation, cells were replica plated onto plates lacking arginine (40), incubated a further 2 days, and scored for growth at the spore colony patch-tester strain intersection. The frequency of recombination in the interval between the two *MAT* loci in MJL1068 was determined by scoring the mating-type phenotype of random spores as previously described (20). A total of 28 of 272 spores were haploid nonmaters; therefore, the map distance in this interval is 10.3 centimorgans.

Sporulation of mass cultures. Saturated overnight cultures in YPD broth (40) were diluted 1/1,000 into 200 ml of SPS broth (31) and grown with vigorous shaking to 3×10^7 to 4×10^7 cells per ml. Cells were harvested by centrifugation, washed once with 1% potassium acetate (prewarmed to 30°C), resuspended at about 1.5×10^7 cells per ml in 400 ml of prewarmed sporulation medium (1% potassium acetate, 0.001% polypropylene glycol 2000 [Aldrich], 6 μg of lysine per ml, 4 μg of arginine per ml, 12 μg of leucine per ml), and aerated with vigorous shaking at 30°C in a 2-liter flask. Samples were removed at intervals for study of commitment to recombination, meiotic divisions, and extraction of the DNA for further analysis.

Commitment to meiotic recombination. Culture aliquots were sonicated briefly to disrupt clumps, diluted, and plated on synthetic medium lacking arginine (40) to select Arg^+ recombinants and onto YPD to measure total viable cells.

Meiotic divisions. Cultures (0.5 to 1 ml) were fixed in 95% ethanol, stained with 4′,6-diamidino-2-phenylindole (Sigma) (40), and examined by fluorescence microscopy. Cells containing two or four nuclei were considered to have completed meiosis I.

DNA extraction. At each time point, a 25-ml aliquot of the sporulating culture was made up to 50% ethanol–25 mM EDTA and stored at −20°C. Cells were harvested by centrifugation, washed once with TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), resuspended in 0.5 ml of 1 M sorbitol–10 mM Na_2PO_4 (pH 7.2)–10 mM EDTA–1% β-mercaptoethanol–1 mg of yeast lytic enzyme (ICN Biochemicals)

per ml, and incubated for 30 min at 37°C. In most experiments, the resulting spheroplasts were harvested by centrifugation, resuspended in 0.5 ml of 50 mM EDTA–0.5% sodium dodecyl sulfate (SDS)–200 μg of proteinase K per ml, and incubated for 30 min at 65°C. Two hundred microliters of 5 M potassium acetate was added, and samples were incubated for 20 min on ice and then were centrifuged at 12,000 × *g* for 30 min at 4°C. Nucleic acids in the supernatant were precipitated with an equal volume of isopropanol. The dried pellet was resuspended in 0.5 ml of TE + RNase (0.2 mg/ml) and incubated for 30 min at 37°C. DNA was precipitated by addition of 0.25 ml of 7.5 M ammonium acetate and 0.75 ml of isopropanol. The precipitate was rinsed with 70% ethanol, dried, and gently resuspended in 0.1 ml of TE. This procedure yielded about 5 μg of DNA. In two experiments (Table 2), proteinase K was used at 400 μg/ml and all incubations were at 37°C.

Analysis of DNA. Standard techniques were used for restriction enzyme digestion, agarose gel electrophoresis, and Southern blot analysis (37). In all but two experiments, heteroduplex DNA molecules containing an *arg4-nsp/ARG4* mismatch were detected as previously described (21) by displaying *Sac*I-*Eco*RV digests of DNA on denaturant gels, blotting gel contents to nylon filters, and hybridizing with a 298-bp *Sac*I-*Eco*RV *ARG4* fragment. In two experiments (Table 2), DNA was digested with *Ear*I and *Dde*I, which yield a 96-bp fragment containing the site of *arg4-nsp*. Digests were displayed on gels containing 15% polyacrylamide (37.5:1, monomer-bisacrylamide) and 30% denaturant, with denaturant and buffers as previously described (11). These gels were run in a Hoeffer SE600 electrophoresis apparatus at 60°C and 200 V until a xylene cyanol dye marker reached the bottom of the gel. Gel contents were electroblotted to nylon membranes and hybridized with radioactive probe prepared by random hexamer priming (37) from the same 96-bp *Ear*I-*Dde*I fragment.

All filters were prehybridized and hybridized with ^{32}P-labelled DNA at 65°C in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]) (37), 1% SDS, 0.05% nonfat dry milk, and 200 μg of denatured carrier DNA per ml. Filters were washed twice at room temperature and twice at 55°C in 0.1× SSPE–0.1% SDS. All washes were for 15 min. Relative levels of the crossover, double-strand break, and heteroduplex fragments were determined by quantitation of the optical density of bands on autoradiograms with the NIH Image program, a gift of Wayne Rasband. Density at positions of fragments of interest was normalized by using the density at the position of the corresponding parental bands on shorter exposures of the same filter.

Calculation of the time of formation of double-strand breaks. The method of Padmore et al. (28) was used. A full description of this method is beyond the scope of this paper; instead, we will simply describe our calculation of the values required by this method.

The frequency of double-strand breaks at a site at each time point was determined as follows. We compared the density of double-strand break bands at each time point with the density of bands produced by crossing over (in lanes containing samples taken late in meiosis) in either the *URA3–arg4-bgl* interval or the *MAT-MAT* interval. The frequency of recombination in these intervals (from genetic determinations) was used to calculate the frequency of double-strand breaks at each time point.

The total fraction of molecules suffering double-strand breaks at a site during meiosis was estimated by using strains that were homozygous for the *rad50-KI81* mutation. The frequency of double-strand breaks in the *DED81-DED82* intergenic region (dsb1 in Fig. 1A) was estimated to be 7% of those of chromosomes (13); the frequency of double-strand breaks near the pBR322-*ARG4* junction (Fig. 2A) was estimated to be 4% of those of chromosomes (48).

By applying the method of Padmore et al. to data from the six experiments summarized in Table 2, we estimated the life span of double-strand breaks to be 1.0 ± 0.4 h. This wide range in values did not substantially affect the subsequent calculation of the median time of appearance of double-strand breaks. The median time of appearance of double-strand breaks estimated by using a double-strand break life span of 1.5 h was only 0.3 h later than that estimated with a life span of 0.5 h.

RESULTS

We extracted DNA from synchronous meiotic cultures and examined it for the presence of structures associated with recombination: double-strand DNA breaks, heteroduplex DNA, and molecules that contained a recombined configuration of parental markers. The time of appearance of these structures was compared with the timing of two landmarks of meiosis, commitment to meiotic levels of recombination and meiotic divisions, which, respectively, represent the earliest genetic signal of meiotic recombination and the stage at which recombination must be completed.

We examined recombination events at two places in the *S. cerevisiae* genome: the *ARG4* locus, and a locus composed of *ARG4* sequences inserted at *MAT* in the context of a pBR322-based plasmid (hereafter referred to as *MAT*::*arg4*). At both loci, the *ARG4* gene was marked with two mutant alleles, *arg4-nsp* and *arg4-bgl*. *arg4-nsp* is a G · C to C · G transversion in the *ARG4* start codon; *arg4-bgl* is a 4-bp fill-in mutation at a *Bgl*II site 1.3 kb downstream of *arg4-nsp* (26). These markers allowed us to determine the time that cells became committed to meiotic levels of recombination (41). Heteroduplex DNA molecules containing an *ARG4/arg4-nsp* mismatch were detected by using denaturant gel electrophoresis (21). Crossovers were detected at the DNA level by using restriction fragment length polymorphisms created by the combination of *arg4-bgl* and other markers, such that crossing over between these markers gave rise to restriction fragments distinguishable from the parental ones. In the following sections, we present two examples of experiments that examined recombination at *ARG4* and at *MAT*::*arg4* and then a summary of results of several replicate experiments.

Timing of recombination events at *ARG4*. The following results are from an experiment using MJL1102. This strain is heterozygous for three markers: *arg4-nsp*, *arg4-bgl*, and *DED81-URA3-DED82*, an insertion of *URA3* sequences upstream of *ARG4* between the *DED81* and *DED82* genes. Recombination between the *URA3* insertion site and *arg4-bgl* creates two new restriction fragments (4.1 and 6.5 kb) distinguishable form the parental ones, which are both 5.3 kb long (Fig. 1A). Since incomplete digestion by *Bgl*II also could produce a 6.5-kb fragment, we measured only levels of the 4.1-kb fragment. Tetrad analysis of MJL1102 indicated that this fragment is produced primarily (in 75% of cases) by crossing over between *URA3* and *arg4-bgl*; the remainder are produced by gene conversion. Of 968 tetrads analyzed, 45 contained a crossover between *URA3* and *arg4-bgl*. Another 15 tetrads contained gene conversion events that would produce the 4.1-kb fragment. Of these 15 tetrads, 11

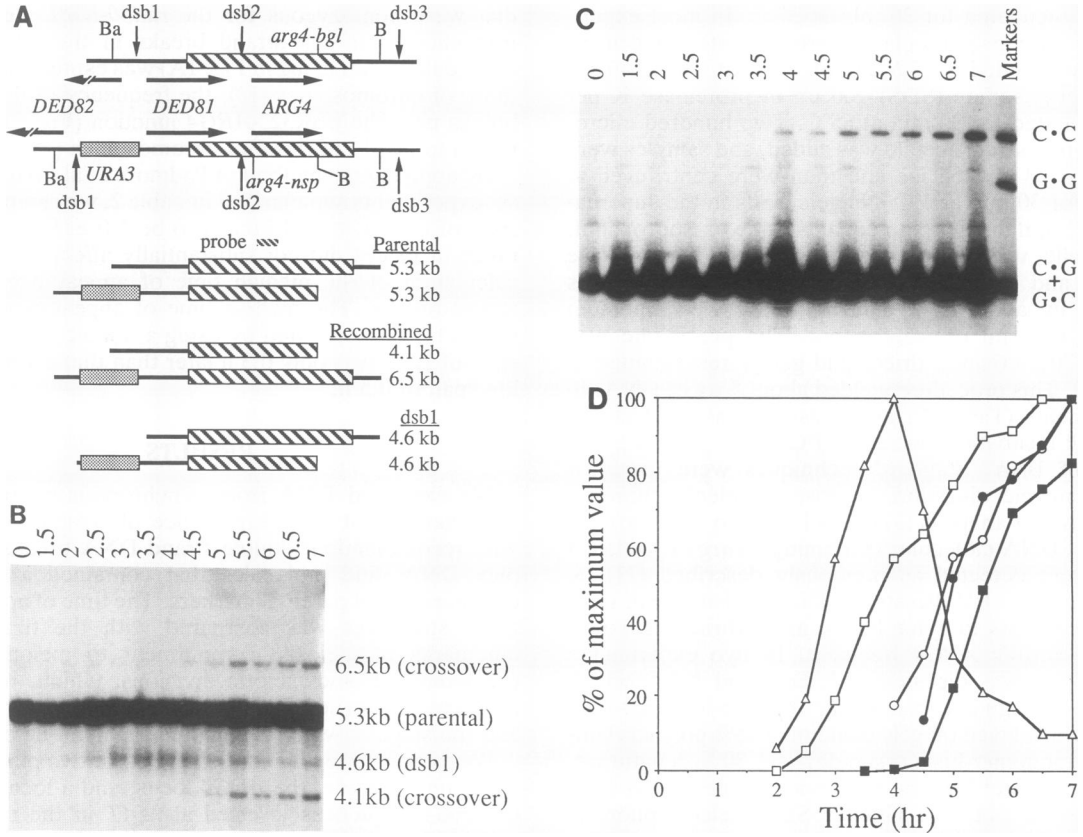

FIG. 1. Timing of double-strand breaks, heteroduplex DNA, and crossing over at *ARG4*. (A) Structure of the *ARG4* region in MJL1102. The interval in which recombination was examined is defined on the left by a *URA3* insertion (shaded box) and on the right by *arg4-bgl*. The position of the *arg4-nsp* mutation is also indicated. Digestion with *Bam*HI (Ba) and *Bgl*II (B) and probing with the 298-bp *Sac*I-*Eco*RV fragment of *ARG4* illuminate restriction fragments from both parents of 5.3 kb; recombination in this interval yields *Bam*HI-*Bgl*II fragments of 6.5 and 4.1 kb. Double-strand breaks occur predominantly at three sites (indicated by vertical arrows) in this region. Double-strand breaks at site 1 (dsb1) are detected in the Southern blot in panel B as a dispersed species with a median size of 4.6 kb. Coding sequences of *DED82*, *DED81*, and *ARG4* are indicated by horizontal arrows. The 3.3-kb *Pst*I fragment used in *MAT*::*arg4* constructs is indicated by a cross-hatched box. (B) Timing of appearance of the products of crossing over and double-strand breaks. DNA was extracted from samples taken at various times after induction of meiosis, digested with *Bam*HI and *Bgl*II, displayed on Southern blots, and probed with the fragment indicated in Panel A. Positions of fragments diagnostic of parental chromosomes, crossover products, and the products of dsb1 are indicated. Fragments diagnostic of dsb2 and dsb3 are not detected in this gel but have been with other combinations of restriction enzymes and probes (13) (Fig. 3). (C) Timing of appearance of heteroduplex DNA containing an *arg4-nsp*/*ARG4* mismatch. *Sac*I-*Eco*RV digests of the same samples were displayed on a uniform denaturant gel, transferred to nylon filters, and probed with the probe indicated in panel A. The marker lane contains a *Sac*I-*Eco*RV digest of an artificial heteroduplex mixture. Positions of homoduplex fragments (C · G + G · C) and of heteroduplex fragments containing C · C and G · G mismatches are indicated. (D) Summary of timing data for MJL1102. Levels are expressed in terms of the maximum value observed. △, double-strand breaks at site 1; □, Arg^+ recombinants; ○, heteroduplex DNA; ●, crossover products; ■, fraction of cells that had completed meiosis I. The final level of Arg^+ recombinants was 4.1×10^{-2} per cell; the maximum level of double-strand breaks was about 4×10^{-2} to 5×10^{-2} per chromosome.

displayed 1:3 Ura^+:Ura^- segregation and 4 displayed 3:1 wild type:mutant segregation for *arg4-bgl*. Five other tetrads displayed 3:1 Ura^+:Ura^- segregation, and four displayed 1:3 wild type:mutant segregation for *arg4-bgl*.

Figure 1B shows the appearance of this recombination diagnostic fragment on Southern blots of DNA extracted at different intervals during meiosis. The 4.1-kb fragment first appeared in samples taken 4.5 h after the induction of meiosis and was present in all subsequent samples. In addition to the two crossover products, a dispersed species with an apparent median size of 4.6 kb was also detected. This species appeared 2 h after the induction of meiosis, reached maximum levels around 4 h, and then gradually disappeared. It is the product of double-strand breaks in sequences between the *DED81* and *DED82* genes, about 2.4 kb upstream of the *ARG4* start codon (43). By comparing the density of this double-strand break band to that of the 4.1-kb crossover product, we estimate that, at 4 h, 4 to 5% of chromosomes are broken at this site. Digests with other restriction enzymes revealed, in agreement with previous reports (43), the presence of second meiosis-induced double-strand break located about 200 bp upstream of the *ARG4* start codon (Fig. 3), as well as an additional break at about +2.9 kb, beyond the 3′ end of the gene (13). All three double-strand breaks appeared and disappeared at similar times (13).

Formation of heteroduplex DNA molecules containing an *ARG4*/*arg4-nsp* mismatch was monitored by using denaturant gel electrophoresis. In this technique, restriction enzyme-digested DNA is displayed on polyacrylamide gels run in conditions such that *ARG4*/*arg4-nsp* heteroduplex DNA

molecules are selectively retarded relative to parental homoduplexes (21, 22). Molecules with a C · C mismatch can be resolved from those with a G · G mismatch. Figure 1C contains a Southern blot of this type of gel, with the same samples whose results are shown in Fig. 1B. Heteroduplex molecules were first visible in samples taken 4 h after transfer to the sporulation medium and were present in all subsequent samples. Only molecules containing C · C mismatches were detected. Since we detected C · C mismatches at less than 20% of their maximum level (Fig. 1D), we conclude that at no time do levels of molecules containing G · G mismatches approach this value.

The timing of appearance of DNA molecules harboring double-strand breaks, C · C mismatches, and crossover products are presented in Fig. 1D, along with the timing of commitment to meiotic levels of recombination and of passage through meiosis I. Commitment to meiotic recombination preceded meiosis I by about 1.5 h. Double-strand breaks appeared at the same time that cells first became committed to meiotic levels of Arg^+ recombinants. Breaks reached a maximum level at 4 h; at that time heteroduplex DNA was first detected. No significant difference between the times of appearance of heteroduplex DNA and of the products of crossing over was observed in this experiments. Both preceded meiosis I by less than an hour.

Timing of recombination events at *MAT*::*arg4*. We also studied meiotic recombination events in strains that contained *ARG4* sequences inserted between a duplication of the *MAT* locus, diagrammed in Fig. 2A. *arg4-bgl*, in association with a natural restriction site polymorphism at *MAT* (a *Bgl*II site exists in *MAT***a** but not in *MAT*α [2]), allows detection of new restriction fragments diagnostic of reciprocal recombination (7). A crossover in the interval defined by the *Bgl*II site in the left-hand copy of *MAT***a** and by *arg4-bgl* (interval A in Fig. 2A) produces two *Bgl*II restriction fragments, one of which (16.3 kb) is distinguishable from parental fragments (>30 and 6.8 kb). Likewise, a crossover in the interval defined by *arg4-bgl* and by the *Bgl*II site in the right-hand copy of *MAT***a** (interval B) produces a new *Bgl*II restriction fragment of 10.8 kb. Fragments diagnostic of crossing over in these two intervals were first detected 4.5 h after initiation of sporulation (Fig. 2B). A dispersed DNA species, with an apparent median size of 13.6 kb, first appeared at 2.5 h, reached highest levels at about 3.5 h, and then gradually disappeared. These fragments are the products of meiosis-induced double-strand breaks in pBR322 sequences about 1.4 kb upstream of the start of *ARG4*. Digests with other restriction enzymes revealed fragments with sizes consistent with those of the products of double-strand breaks at this site (48) (Fig. 3). By comparing the density of this double-strand break band with that of the 15.8- and 10.3-kb recombination products, we estimate that, at 3.5 h, about 1% of chromosomes are broken at this site.

As was mentioned above, a double-strand break occurs during meiosis at the normal *ARG4* locus, in promoter sequences ca. 200 bp upstream of the start of *ARG4* (43). We did not detect significant amounts of fragments of the size expected for a double-strand break at the *ARG4* promoter contained in the *ARG4* insert at *MAT*, even though the 3.3-kb *ARG4* fragment inserted at *MAT* contains these sequences and flanking sequences for at least 1.2 kb on either side. A comparison of the position and amount of double-strand breaks at the normal *ARG4* locus and in the *MAT*::*arg4* insert is presented in Fig. 3. Despite the absence of detectable levels of breaks in *arg4* sequences inserted at *MAT*, the frequency of Arg^+ recombinants produced by diploids with *arg4-nsp* and *arg4-bgl* located at *MAT*::*arg4* (4.7×10^{-2} per cell) was similar to that observed in MJL1102, in which *arg4-nsp* and *arg4-bgl* are present at the normal *ARG4* locus (4.1×10^{-2} per cell).

The time of appearance of heteroduplex DNA molecules containing an *arg4-nsp*/*ARG4* mismatch was also determined, by using the approach described above for the study of recombination events at the normal *ARG4* locus. Times of appearance of molecules containing double-strand breaks, C · C mismatches, and the products of crossing over in the interval that contains *arg4-nsp* (interval A) are presented in Fig. 2C, along with the timing of commitment to meiotic recombination and of first meiotic division. As was seen at the normal *ARG4* locus, molecules containing double-strand breaks appeared early in meiotic prophase, at about the same time cells first displayed commitment to meiotic levels of Arg^+ recombinants. Heteroduplex DNA molecules containing an *arg4-nsp*/*ARG4* mismatch and DNA molecules diagnostic of crossing over both appeared late in meiotic prophase, shortly before the onset of the first meiotic division.

Timing of meiotic events as determined in several replicate experiments. We performed, in addition to the experiments presented in detail above, four other meiotic time course experiments. Three examined meiotic recombination at the normal *ARG4* locus; a fourth examined recombination at *MAT*::*arg4*. The median time of appearance (time of half-maximum level) of commitment to Arg^+ recombinants, heteroduplex DNA, recombined DNA molecules, and cells completing meiosis I are presented in Table 2 and Fig. 4.

In agreement with previous findings (28), the products of crossing over did not appear until late in meiotic prophase, 0.4 h before completion of meiosis I. Crossover products appeared, on the average, 0.3 h after heteroduplex DNA. Although overlap exists in the distributions of median times of appearance of heteroduplex DNA and crossover products, heteroduplex molecules always appeared at the same time or earlier than did crossovers. We were concerned that the extraction procedure (which included a 65°C incubation) and the fragment used to detect heteroduplex molecules (which was 298 bp long) might preclude recovery and detection of intermediates that contain short regions of heteroduplex DNA. Therefore, in the two experiments indicated as MJL1102[d] in Table 2, DNA was extracted at a lower temperature (37°C rather than 65°C), and a small fragment (only 98 bp in length) was used to detect heteroduplex DNA. We observed little difference between the relative time of appearance of heteroduplex and crossover products in these experiments and the relative times determined in others.

Double-strand breaks reached a maximum level at about the same time that half-maximum levels of commitment to meiotic recombination were attained, and the highest level of double-strand breaks occurred about 0.9 h before the median time of appearance of heteroduplex DNA. Unlike the other parameters measured, double-strand breaks are transient. Therefore, the level of breaks at a given time will reflect not only the portion of the culture that has experienced breakage but also the portion in which breaks either have been processed to form recombination intermediates or have been fully repaired. Padmore et al. (28) describe a method to derive a cumulative curve for appearance of double-strand breaks from noncumulative data. Using this method, we estimate that the median time of appearance of double-strand breaks was 1.8 h (± 0.1 h) earlier that the median time of passage through meiosis I, 1.4 h (± 0.2 h) earlier than the median time of appearance of recombined DNA molecules,

and 1.1 h (± 0.2 h) earlier than the median time of appearance of heteroduplex DNA. These values are in good agreement with those obtained by comparing the time of maximum double-strand break levels with the median times of completion of meiosis I (1.6 ± 0.3 h), of appearance of recombined DNA (1.2 ± 0.3 h), and of appearance of heteroduplex DNA (0.9 ± 0.3 h). They are also in general agreement with other studies of the timing of double-strand breaks and crossing over during meiosis (8, 12, 28, 43).

DISCUSSION

Stable heteroduplex DNA and crossover products both appear late in meiotic prophase. We have determined the time of formation of stable heteroduplex DNA molecules (signals of recombination intermediates) relative to the time of appearance of recombined DNA molecules (a signal of the resolution of these intermediates). Both heteroduplex and recombined DNA molecules appeared late in meiotic prophase, shortly before the first meiotic nuclear division, and only a short period separated their times of appearance. A similar temporal relationship between heteroduplex formation and the first meiotic division has been found in a study of meiotic recombination at the *HIS4* locus (24). As will be discussed below, these findings have implications regarding the nature of hybrid DNA structures that might play a role in chromosome pairing during meiosis.

Although current models of meiotic recombination differ in detail, most contain three common features: first, the formation of initiating lesions; second, the processing of lesions to form intermediate structures containing heteroduplex DNA; and third, the resolution of intermediates to form

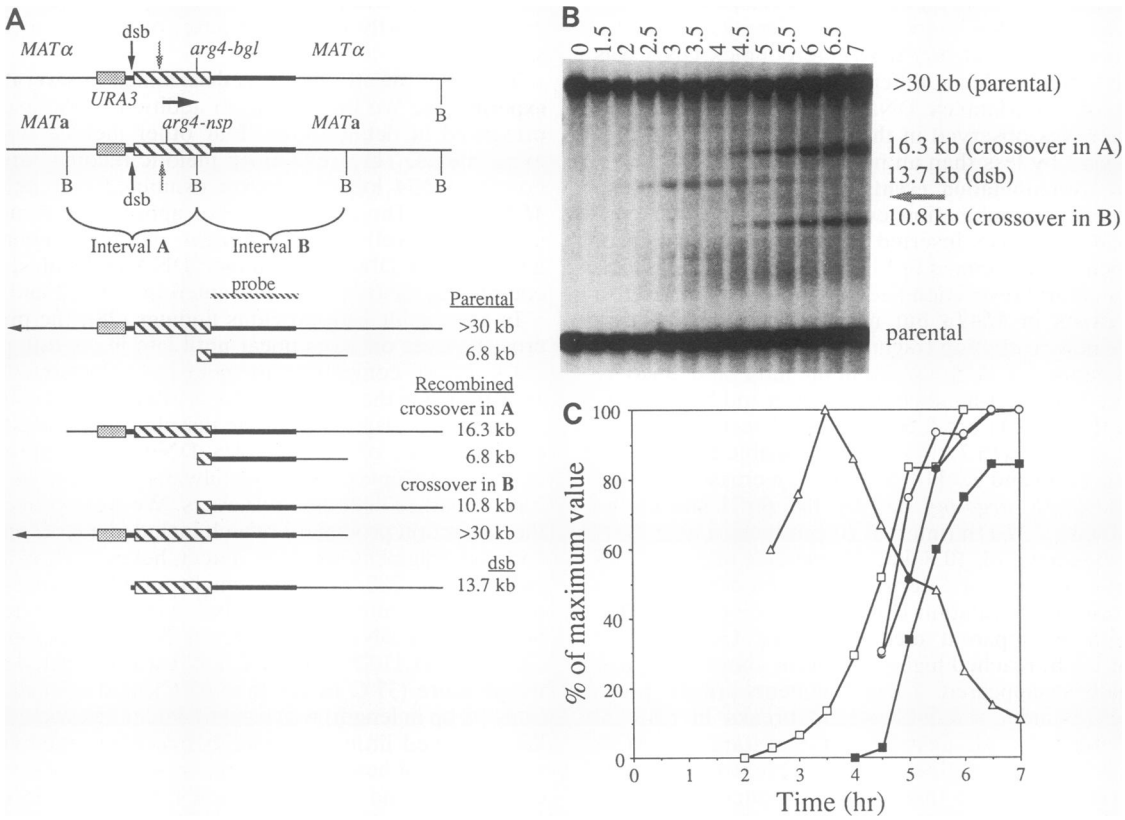

FIG. 2. Timing of double-strand breaks, heteroduplex DNA, and crossing over at *MAT*::*arg4*. (A) Structure of the *MAT*::*arg4* region of MJL1068 and MJL1124. A nontandem duplication of the *MAT* locus flanks a pBR322 (thick lines) insert containing the *URA3* gene (shaded box) and a 3.3-kb *Pst*I fragment (cross hatched box) containing mutant copies of *ARG4* marked with *arg4-nsp* or *arg4-bgl*. *ARG4* coding sequences are indicated by a horizontal arrow. The interval in which recombination was examined (Interval A) is defined on the left by a *Bgl*II restriction site polymorphism between *MAT***a** and *MAT*α and on the right by *arg4-bgl*. Digestion with *Bgl*II and probing with a pBR322 derivative lacking sequences between the *Hin*dIII and *Bam*HI sites reveals parental fragments of 6.8 and >30 kb; crossing over in interval A produces a novel (*MAT***a** *arg4-bgl MAT*α) fragment of 16.3 kb detected by this probe; crossing over in the adjacent interval B produces a novel fragment of 10.8 kb. Also shown is the size of fragments detected by the probe that are produced by double-strand breaks in the region of pBR322 immediately to the left of the *ARG4* insert. A double-strand break in the *ARG4* promoter region (shaded vertical arrow) analogous to dsb2 in Fig. 1A would produce a fragment of about 12.5 kb. (B) Timing of appearance of the products of crossing over and double-strand breaks at the *MAT*::*arg4* locus of MJL1124. DNA was extracted from samples taken at various times after induction of meiosis, digested with *Bgl*II, displayed on Southern blots, and probed with the fragment indicated in panel A. Positions of fragments diagnostic of parental chromosomes, crossing over in intervals A and B, and the products of double-strand breaks are indicated. The expected position of fragments produced by a double-strand break in the *ARG4* promoter region is indicated by a shaded arrow. The faint band appearing just below this position is produced by either gene conversion of *arg4-bgl* to wild-type or events in which crossovers occurred in both intervals A and B (13). (C) Summary of the timing of meiotic events in MJL1124. △, double-strand breaks; □, Arg^+ recombinants; ○, heteroduplex DNA; ●, products of crossing over in interval A; ■, fraction of cells that had completed meiosis I. The final level of Arg^+ recombinants was 4.7×10^{-2} per cell; the maximum level of double-strand breaks was 9×10^{-3} per chromosome.

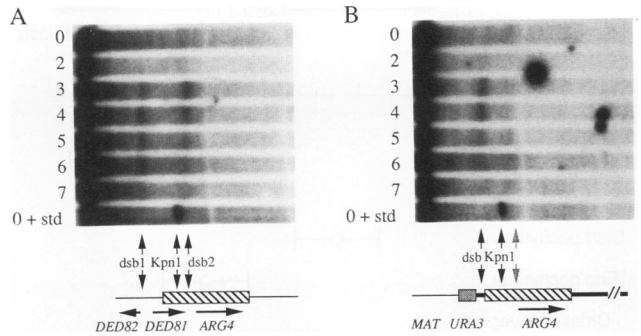

FIG. 3. Comparison of double-strand breaks at the normal *ARG4* locus and at *MAT*::*arg4* in strain MJL1068. DNA samples taken at various times during meiosis were digested with *Xba*I and displayed on Southern blots. In addition, a small aliquot of the 0-h sample was digested with *Xba*I and *Kpn*I and mixed with *Xba*I-digested 0-h DNA. This sample (0 + std) served as an internal standard to allow comparison of the location and amount of double-strand breaks at *MAT*::*arg4* and at the normal *ARG4* locus. A *Kpn*I site is located 0.6 kb upstream of the start of the *ARG4* gene at both loci. (A) Double-strand breaks at the normal *ARG4* locus. Arrows indicate the positions of dsb1 and dsb2 (Fig. 1A) relative to the *Kpn*I site. A *Pst*I-*Sna*BI fragment located 3.5 to 4.8 kb downstream of the *ARG4* translation start site was used as a probe. *Xba*I sites are located about 6.1 kb downstream and about 7.4 kb upstream of the *ARG4* translation start site (13). Coding sequences and the 3.3-kb *Pst*I fragment used in *MAT*::*arg4* constructs are indicated as in Fig. 1A. (B) Double-strand breaks at *MAT*::*arg4*. The same filter was stripped and reprobed with the 0.8-kb *Pst*I-*Eco*RI fragment of pBR322. Arrows indicate the position of the single major double-strand break in this construct (Fig. 2A) relative to the *Kpn*I site. Shaded arrows indicate the expected position of a double-strand break in the *ARG4* promoter region. Other features are indicated as in Fig. 2A. *Xba*I sites are located in the *MAT* sequences flanking the insert, 5.7 kb downstream and 5.1 kb upstream of the *ARG4* translation start site.

mature recombinant duplex molecules. If one assumes that double-strand DNA breaks initiate meiotic recombination, then one might expect heteroduplex-containing DNA molecules to appear soon after double-strand breaks and to persist until the time that mature crossover molecules are formed. The results of experiments reported in this paper do not support this expectation. Heteroduplex-containing molecules were not detected until late in meiosis, and the time separating heteroduplex-containing and mature crossover molecules was modest, approaching the limits of experimental error. Nevertheless, we believe that heteroduplex DNA molecules are formed before mature crossover molecules, since in all experiments in which appearance of both was monitored, heteroduplex DNA appeared before crossover products. More important, however, is the fact that the time separating appearance of heteroduplex DNA from the formation of mature crossover products was short (about 0.3 h) relative to the time separating appearance of double-strand DNA breaks from the appearance of mature crossover products (about 1.2 h). If double-strand breaks initiate meiotic recombination or occur at the same time as other lesions that initiate meiotic recombination, then a gap of about 1 h exists between the time of formation of initiating lesions and the time that parental molecules are stably joined by Watson-Crick base pairing. It should be noted that a similar delay has been observed in studies of induced mitotic recombination (10, 29, 35, 36, 47).

One way to account for the failure to detect heteroduplex-containing DNA molecules until late in meiotic prophase would be to suggest that hybrid DNA is present early in meiosis, but in structures that cannot be detected on denaturant gels. For example, if the average length of heteroduplex DNA in early intermediates was less than the length of the restriction fragment used in our analysis, fragments containing heteroduplex DNA would usually also contain single-strand interruptions or branched structures and would not migrate as a discrete species in denaturant gels. Since 96-bp-long heteroduplex DNA molecules appeared at about the same time as did 298-bp heteroduplex fragments (Table 2), the above suggestion could only be true if the average length of heteroduplex DNA in these intermediates was short, on the order of 150 bp or less. This distance is considerably shorter than either the average coconversion tract length observed at *ARG4* (39) or the average length of single-strand DNA at the ends of double-strand break fragments (44). Another way to account for the failure to detect heteroduplex-containing DNA until late in meiotic prophase would be to suggest that hybrid DNA is present earlier in a form in which parental contributions are joined in unstable or nonduplex structures that would not be detected on denaturant gels (see below).

TABLE 2. Timing of events relative to meiosis I

Strain	Time (h) for following event:				
	Meiosis I[a]	Commitment to recombination[b]	Double-strand break[c]	Heteroduplex DNA[b]	Crossing over[b]
ARG4					
MJL1102	5.5	−1.8	−1.5	−0.8	−0.6
MJL1102	6.1	−1.6	−1.1	−0.4	ND
MJL1102[d]	5.4	ND	−1.4	−0.8	−0.2
MJL1102[d]	5.5	−1.3	−1.9	−0.6	−0.2
MAT::*arg4*					
MJL1068	5.3	−0.8	−1.7	−0.8	−0.6
MJL1124	5.3	−0.9	−1.8	−0.6	−0.4
Avg ± SD		−1.3 ± −0.4	−1.6 ± −0.3	−0.7 ± −0.2	−0.4 ± −0.2

[a] Time after transfer to sporulation medium at which half the cells had completed the first meiotic division (median time of completion of meiosis I).
[b] Time, relative to median time of completion of meiosis I, at which half the maximum level was attained (median time of appearance). ND, not determined.
[c] Time, relative to median time of completion of meiosis I, at which the maximum level was attained.
[d] Spheroplasts were lysed at 37°C, and a 98-bp fragment was examined for the presence of heteroduplex DNA (see Materials and Methods).

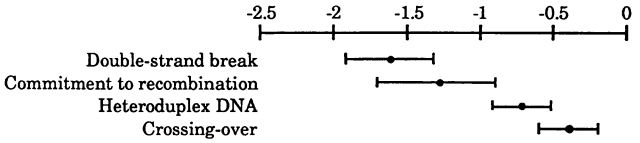

FIG. 4. Timing of events relative to meiosis I. Graphic representation of mean values ± standard deviations from Table 2.

Is meiotic chromosome pairing effected by recombination? Padmore et al. (28) examined the temporal relationship between the formation of meiosis-induced double-strand breaks, the pairing of homologs by synaptonemal complex (SC), and the formation of mature recombinant molecules. They found that double-strand breaks appeared at the same time as did early SC precursors, prior to the pachytene stage of meiosis, in which chromosomes are fully synapsed. Mature recombinant products appeared at or just after the end of pachytene, and the pachytene stage occupied a substantial portion (1.3 of 2.3 h) of the period between the time of appearance of breaks and the time of appearance of recombined molecules. Given our observation that stable heteroduplex DNA is not formed until just before (0.3 h) mature recombinant products appear, what is the likelihood that chromosome synapsis is effected by the formation of heteroduplex DNA?

The sporulation procedure used by Padmore et al. (28) differs from that used here. As a result, the net time from inoculation in sporulation medium to completion of meiosis I differs significantly between the two works (5.5 ± 0.3 h here versus 8.1 ± 0.1 h in Padmore et al.). To compare the two sets of data, we have focused on the temporal distribution of events during the period bounded by the time of appearance of double-strand breaks (the first physical signal of meiotic recombination) and the time of completion of the first meiotic nuclear division (when recombination between homologs must be complete). This comparison, described below, is summarized in Fig. 5. Although the period between these two temporal landmarks was considerably shorter in our experiments (1.8 ± 0.1 h) than in those of Padmore et al. (3.8 ± 0.3 h), mature recombinant molecules appeared at the same relative time (about 75 to 80% of the total time transpired between double-strand breaks and meiosis I) in both sets of experiments (Fig. 5). Calculation of the relative times of appearance of other events (stable heteroduplex DNA, beginning and end of the pachytene stage) indicates that stable heteroduplex DNA molecules are not formed until the end of pachytene (both about 60% of the time transpired in the period) and that homolog synapsis is complete (beginning of pachytene, at about 25 to 30% of the time transpired) well before the time that stable hereroduplex DNA molecules appear. Thus, if one assumes that pachytene occupied the similar portions of the double-strand break-meiosis I interval in our experiments and in the experiments of Padmore et al., one is forced to conclude that formation of stable heteroduplex DNA cannot be responsible for the homolog pairing.

An extreme interpretation of these findings would suggest that the recognition of homology necessary for chromosome pairing does not occur via the formation of recombination intermediates and that the entire recombination reaction does not occur until chromosomes are stably paired. As an alternative, we suggest that early stages of chromosome pairing are effected by recombination intermediates that either are unstable or do not involve standard Watson-Crick

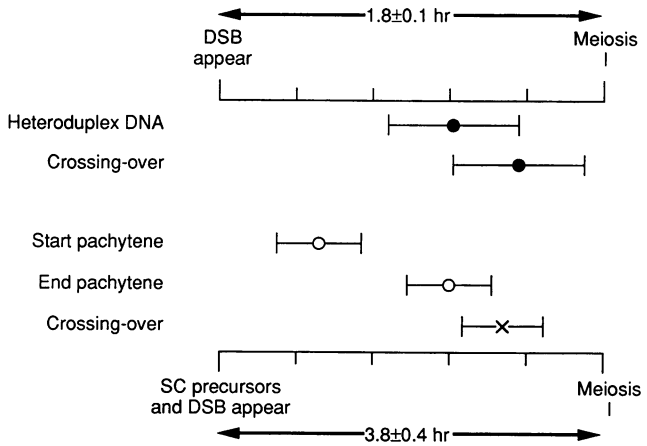

FIG. 5. Comparison of the timing of meiotic events as determined by this work and by Padmore et al. (28). To facilitate comparison, we have equalized the interval between appearance of double-strand breaks and completion of meiosis I as determined in this work and the interval between appearance of SC precursors and completion of meiosis I (telophase I) as presented in Table 2 of Padmore et al. (28). Padmore et al. have shown that double-strand breaks and SC precursors appear contemporaneously. ●, relative times of appearance for heteroduplex DNA and crossover products as determined in this work; ○, relative times of appearance and disappearance of nuclei with fully synapsed homolog (pachytene stage) as presented in Table 2 (summary of data for time courses 10, 11, and 12) of Padmore et al. (28); ×, relative time of appearance of crossover products as determined from Fig. 6 and 7 (data for time course 11 only) of Padmore et al. (28). Error bars for each event reflect the error of measurements of the individual event plus the indicated error in measurement of the length of the double-strand break (DSB)-meiosis I interval.

base pairing. For example, pairing of DNA molecules initially might occur in the context of protein and/or RNA complexes that stabilize otherwise unstable structures, such as paranemic joints or structures (such as Holliday junctions containing nicks on both chromosomes) that could be destroyed by branch migration. Removal of protein and RNA during DNA purification would result in the loss of such structures. Alternatively, initial pairing might occur by the formation of triplex DNA structures, which would not be detected by the denaturant gel system used in this work. Both paranemic joints and triplex DNA have been observed among the products of in vitro strand transfer reactions (5, 16, 30, 45).

The use of such unstable or reversible structures during the initial stages of chromosome pairing could provide the cell with a means to eliminate pairing events that involve short regions of sequence similarity located on nonhomologous chromosomes (9, 18). These fortuitous associations could be detected and dissolved during the early stages of chromosome synapsis. Pairing between regions with more extensive homology would be maintained by components of the recombination machinery until chromosome synapsis was complete, at which point an intracellular signal would trigger the conversion of these unstable associations first to stable intermediates containing heteroduplex DNA and then to mature recombined duplex DNA molecules. This hypothesis, if correct, predicts that the earliest intermediates in recombination and chromosome pairing would be detected only through the use of reagents (such as intra- or interstrand cross-linkers) that confer stability in the absence of protein

and/or RNA components of the meiotic recombination machinery.

Mismatch correction occurs during meiotic prophase. Heteroduplex DNA molecules with an *arg4-nsp/ARG4* mismatch can contain either C · C or G · G. In a previous study, we showed that molecules with G · G mismatches are formed but are efficiently repaired by a *PMS1*-dependent mismatch repair system prior to spore maturation (21). In the experiments reported here, DNA extracted from cells in meiotic prophase contained only C · C mismatches. Our failure to detect G · G mismatches at any time during meiosis is consistent with the suggestion that they are corrected soon after they are formed. If G · G mismatches survive for a significant time during meiosis, they must be present in structures that are not detected by our assay (see above). Correction of these mismatches must occur before recombination intermediates are resolved, and therefore must occur before meiosis I.

Chromosomal environment influences the susceptibility of a site to meiosis-induced cleavage. In the experiments reported here, double-strand breaks appeared at about the same time that cells become committed to perform meiotic levels of recombination, a finding consistent with the suggestion that formation of double-strand breaks is the first irreversible step in the meiotic recombination pathway. Although the precise relationship between meiosis-induced double-strand breaks and meiotic recombination remains to be established, our findings do indicate that the formation of double-strand breaks during meiosis does not simply involve recognition and cutting of a short nucleotide sequence. Sequences in the promoter region of *ARG4* are subject to a meiosis-induced double-strand break when present at the normal *ARG4* locus (43). This same region did not display detectable levels of double-strand breaks when present in an *arg4* insert at *MAT*, even though that insert contained sequences from the normal *ARG4* locus for about 1.2 kb to the left of the promoter region and 2 kb to the right. Instead, breaks were observed in vector sequences, sequences that are not normally found in the yeast genome.

One way to account for this result would be to suggest that the insertion of *arg4* sequences at *MAT* in the context of a pBR322-based plasmid leads to an alteration of chromatin structure in the promoter region, so that it is not accessible to the endonuclease responsible for meiosis-induced cleavage. Alternatively, the total level of breaks in a region might be limited by some factor other than accessibility. Thus, if the novel double-strand break site near the pBR322-*ARG4* junction in the *MAT*::*ARG4* region was frequently recognized and cut, nearby sites (such as the *ARG4* promoter) might be cut less often. Although our results do not distinguish between these and other possible explanations, they do indicate that the systems that determine whether or not a site is cleaved during meiosis must act over considerable distances.

ACKNOWLEDGMENTS

We thank Eric Alani, Liang Cao, Ruth Padmore, and Nancy Kleckner for yeast strains, for communicating results in advance of publication, and for advice on living happily with SK1. We also thank Dilip Nag and Tom Petes for communicating results in advance of publication. We thank Neil Schultes, Alain Nicolas, Doug Treco, and Jack Szostak for plasmids that contained sequences in the vicinity of *ARG4*. We also thank Carol Wu for her help in constructing some of the yeast strains used in this study and Chris Szent-Gyorgyi for suggesting the use of polypropylene glycol 2000 as an anticlumping agent. We also thank Dhruba Chattoraj, Jim Haber, Angelos Kalogeropoulos, Claude Klee, James McCarthy, Alain Nicolas, Anca Segall, and Michael Yarmolinsky for suggesting improvements to the manuscript.

REFERENCES

1. **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.
2. **Astell, C. R., L. Ahlstrom-Jonasson, M. Smith, K. Tatchell, K. A. Nasmyth, and B. D. Hall.** 1981. The sequence of DNAs coding for the mating-type loci of *Saccharomyces cerevisiae*. Cell **27**:15–23.
3. **Beacham, I. R., B. W. Schweitzer, H. M. Warrick, and J. Carbon.** 1984. The nucleotide sequence of the yeast *ARG4* gene. Gene **29**:271–279.
4. **Bell, L. R., and B. Byers.** 1983. Homologous association of chromosomal DNA during yeast meiosis. Cold Spring Harbor Symp. Quant. Biol. **47**:829–840.
5. **Bianchi, M., C. DasGupta, and C. M. Radding.** 1983. Synapsis and formation of paranemic joints by *E. coli* RecA protein. Cell **34**:931–939.
6. **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.
7. **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.
8. **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.
9. **Carpenter, A. T. C.** 1987. Gene conversion, recombination nodules, and the initiation of meiotic synapsis. Bioessays **6**:232–236.
10. **Connoly, B., C. I. White, and J. E. Haber.** 1988. Physical monitoring of mating-type switching in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:2342–2349.
11. **Fischer, S. G., and L. S. Lerman.** 1983. DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. Proc. Natl. Acad. Sci. USA **80**:1579–1583.
12. **Game, J. C., K. C. Sitney, V. E. Cook, and R. K. Mortimer.** 1989. Use of a ring chromosome and pulsed-field gels to study interhomolog recombination, double-strand DNA breaks and sister-chromatid exchange in yeast. Genetics **123**:695–713.
13. **Goyon, C., and M. Lichten.** Unpublished observations.
14. **Hastings, P. J.** 1987. Meiotic recombination interpreted as heteroduplex correction. p. 107–137. *In* P. O. Moens (ed.), Meiosis. Academic Press, Inc., Orlando, Fla.
15. **Hastings, P. J.** 1987. Models of heteroduplex formation, p. 139–156. *In* P. B. Moens (ed.), Meiosis. Academic Press, Inc., Orlando, Fla.
16. **Hsieh, P., C. S. Camerini-Otero, and R. D. Camerini-Otero.** 1990. Pairing of homologous DNA sequences by protein: evidence for three-stranded DNA. Genes Dev. **4**:1951–1963.
17. **Kane, S., and R. Roth.** 1974. Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118**:8–14.
18. **Kleckner, N., R. Padmore, and D. K. Bishop.** Meiotic chromosome metabolism: one view. Cold Spring Harbor Symp. Quant. Biol. **56**:729–743.
19. **Kolodkin, A. L., A. J. S. Klar, and F. W. Stahl.** 1986. Double-strand breaks can initiate meiotic recombination in *S. cerevisiae*. Cell **46**:733–740.
20. **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.
21. **Lichten, M., C. Goyon, N. P. Schultes, D. Treco, J. W. Szostak, J. E. Haber, and A. Nicolas.** 1990. Detection of heteroduplex DNA molecules among the products of *Saccharomyces cerevisiae* meiosis. Proc. Natl. Acad. Sci. USA **87**:7653–7657.
22. **Myers, R. M., N. Lumelsky, L. S. Lerman, and T. Maniatis.** 1985. Detection of single base substitutions in total genomic

DNA. Nature (London) **313**:495–498.
23. **Nag, D. K., and T. D. Petes.** 1990. Genetic evidence for preferential strand transfer during meiotic recombination in yeast. Genetics **125**:753–761.
24. **Nag, D. K., and T. D. Petes.** Personal communication.
25. **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.
26. **Nicolas, A., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature (London) **338**:35–39.
27. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78**:6354–6358.
28. **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.
29. **Plessis, A., A. Perrin, J. E. Haber, and B. Dujon.** 1992. Site-specific recombination determined by I-*Sce*I, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. Genetics **130**:451–460.
30. **Rao, B. J., M. Dutreix, and C. M. Radding.** 1991. A stable three-stranded DNA intermediate made by RecA protein. Proc. Natl. Acad. Sci. USA **88**:2984–2988.
31. **Resnick, M. A., S. Stasiewicz, and J. C. Game.** 1983. Meiotic DNA metabolism in wild-type and excision-deficient yeast following UV exposure. Genetics **104**:583–601.
32. **Roeder, G. S.** 1990. Chromosome synapsis and genetic recombination: their roles in meiotic chromosome segregation. Trends Genet. **6**:385–389.
33. **Rose, M., P. Grisafi, and D. Botstein.** 1984. Structure and function of the yeast *URA3* gene. Gene **29**:113–124.
34. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
35. **Rudin, N., and J. E. Haber.** 1988. Efficient repair of *HO*-induced chromosomal breaks in *Saccharomyces cerevisiae* by recombination between flanking homologous sequences. Mol. Cell. Biol. **8**:3918–3928.
36. **Rudin, N., E. Sugarman, and J. E. Haber.** 1989. Genetic and physical analysis of double-strand break repair and recombination in *Saccharomyces cerevisiae*. Genetics **122**:519–534.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
38. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
39. **Schultes, N. P., and J. W. Szostak.** 1990. Decreasing gradients of gene conversion on both sides of the initiation site for meiotic recombination at the *ARG4* locus in yeast. Genetics **126**:813–822.
40. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Sherman, F., and H. Roman.** 1963. Evidence for two types of allelic recombination in yeast. Genetics **48**:255–261.
42. **Smithies, O., and P. Powers.** 1986. Gene conversions and their relation to homologous chromosome pairing. Philos. Trans. R. Soc. Lond. B **312**:291–302.
43. **Sun, H., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. Double-strand breaks at an initiation site for meiotic gene conversion. Nature (London) **338**:87–90.
44. **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.
45. **Umlauf, S. W., M. M. Cox, and R. B. Inman.** 1990. Triple-helical pairing intermediates formed by RecA protein. J. Biol. Chem. **265**:16898–16912.
46. **Vandeyar, M. A., M. P. Weiner, C. J. Hutton, and C. A. Batt.** 1988. A simple and rapid method for the selection of oligodeoxynucleotide-directed mutants. Gene **65**:129–133.
47. **White, C. I., and J. E. Haber.** 1990. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. **9**:603–610.
48. **Wu, T.-C., and M. Lichten.** Unpublished data.