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Initiation of meiotic recombination is independent of interhomologue interactions

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ABSTRACT **In yeast meiosis, crossing-over between homologues is dependent upon double-strand breaks. We demonstrate that the occurrence of these breaks is independent of pairing between homologues by showing that they occur with normal frequency, timing, and position in the absence of a homologue. This observation supports models that view double-strand breaks as initiating events and crossing-over as a consequence of repair of these breaks.**

As eukaryotic cells enter meiosis, they replicate their DNA and then enter a prolonged prophase, during which their chromosomes condense, synapse, and recombine. During meiosis in yeast, the rate of interhomologue genetic exchange is induced several thousandfold over mitotic recombination levels, while sister chromatid exchange is induced to a lesser extent (for review, see ref. 1). The meiotic mechanism that favors exchange between homologues over that between sister chromatids is not known.

Recent studies have indicated that a significant fraction of meiotic homologous recombination (gene conversion and reciprocal exchange) is initiated at specific sites in the yeast *Saccharomyces cerevisiae* (2–6). One such site has been identified near the *ARG4* transcription promoter (2). Gel electrophoresis of restriction fragments of DNA from meiotic cells has shown that meiosis-specific double-strand breaks (DSBs) occur at this site as well as at other meiotic recombination hot spots (3, 6, 7). The timing of these DSBs is consistent with their postulated role as initiators in the recombination process, and they are dependent on early meiotic recombination functions [e.g., *RAD50* and *SPO11* (3, 7)]. A recent comparison between the positions of meiosis-specific DSBs and genetic distance in two regions of the yeast genome led Wu and Lichten (4) to conclude that "most, if not all, meiotic recombination is initiated by promoter-associated DSBs."

The mechanism by which chromosomes find their homologues is not understood. One possibility is that the search process is driven by the recombinational repair of such DSBs that are induced in meiosis. This postulated early role for DSBs is supported by the findings that DSBs precede the formation of the synaptonemal complex (SC), a tripartite structure that connects paired condensed homologs along their entire lengths (8), and that DSBs occur in mutant strains defective in SC formation (3, 9).

Another possibility, suggested in a recent model (refs. 8, 10, and 11; for review, see ref. 12), proposes that a homology search precedes DSBs. In this model, unstable paranemic interaction involving two intact duplex homologues may allow the homologues to recognize and align with one another. The unstable alignments may then be stabilized when DSBs occur and are processed, producing 3′-single-stranded tails capable of initiating plectonemic interactions (10, 11). This model is supported by temporal relationships of chromosome pairing, recombination, and the formation of SC in meiotic prophase (8, 11, 13, 14).

The recent identification of homologous associations very early in meiosis (11, 14) and the temporal relationships explicit in the second model suggest that homologue recognition occurs before, and may be required for, the formation of DSBs. We test this functional dependency by asking whether meiotic DSBs occur normally in the absence of a homologue.

MATERIALS AND METHODS

Strains and Media. Yeast strains were all isogenic SK1 strains. SK1 strains undergo a rapid and synchronous meiosis (15). F1031 is *MAT***a**, *ho::LYS2, lys2, ura3, leu2::hisG, rad50s::URA3, sir3::LEU2*. F1032 is identical, except that it is a *MAT***a**/*MAT*α *SIR3/SIR3* diploid. F1007 is identical to F1032, except that it is heterozygous for a 5.1-kb deletion encompassing the *ARG4* gene (shown in Fig. 1). These strains were transformed to make the *rad50S::URA3* allele using pNKY349 (18). In a *rad50S* strain, meiosis-specific DSBs occur but are not repaired (18). F1031 was transformed with a *sir3::LEU2* allele by one-step transplacement using pJR841 (19) (obtained from Margit Foss, University of California-Berkeley). The 5.28-kb deletion was generated by two-step transplacement (20) using YIp5-arg4Δ, a plasmid generated by subcloning an 8.0-kb *Bam*HI–*Xho* I fragment containing *ARG4* from pSPO13-1 (21) into YIp5 (22), followed by deletion of the 5.1-kb *Sna*BI–*Eco*47III fragment.

Yeast medium YEP [1% yeast extract/2% (wt/vol) Bacto Peptone] was supplemented with either 2% (vol/vol) glycerol (YEPG) or 2% (wt/vol) raffinose (YEPR). The sporulation medium (SPM) was 1% potassium acetate/0.1% raffinose.

Sporulation Regimen. Cells of strains F1007, F1031, and F1032 were first grown in YEPG medium overnight, diluted, and then grown in YEPR medium overnight. Cells were then diluted to approximately 10^7 cells per ml and transferred to SPM to induce meiosis. The first YEPG growth step was included to select against petite mutants, which are incapable of entering meiosis. When the cells were transferred to SPM, the population consisted of >95% large unbudded cells. Aliquots of 25 ml were removed every hour for 10 h.

DNA Extraction and Southern Blot Analysis. After each 25-ml aliquot was taken from the sporulating cultures, the cells were immediately pelleted, resuspended in 1.0 ml of ice-cold 70% EtOH/10 mM EDTA, transferred to a 1.5-ml Eppendorf tube, and stored at −70°C. Genomic DNA was prepared from these cells as described (9). Approximately 0.25 μg of genomic DNA was digested with *Bam*HI and *Bgl* II simultaneously (for positions of these sites, see Fig. 1) and subjected to electrophoresis on a 0.7% agarose gel in TAE buffer (40 mM Tris acetate/1 mM EDTA) for 2.5 h at 100 V. The gel was then soaked in 0.2 M HCl for 15 min, 0.5 M NaOH for 45 min, and 1 M Tris·HCl (pH 7.0) for 45 min. DNA

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Abbreviations: DSB, double strand break; SC, synaptonemal complex.

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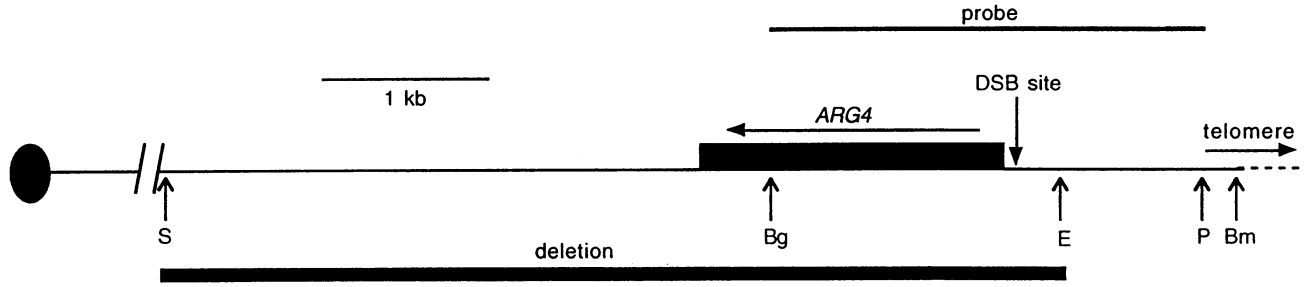

FIG. 1. Region of chromosome VIII showing *ARG4* and flanking regions. The position of the meiosis-specific DSB site is approximately −185 bp relative to beginning of the *ARG4* coding region (16). Relevant restriction sites are Bg (*Bgl* II), Bm (*Bam*HI), S (*Sna*BI), P (*Pst* I), and E (*Eco*47III). In the deletion heterozygote diploid F1007, one of the parents has a deletion extending from the *Eco*47III site to the *Sna*BI site. A 2.68-kb *Pst* I–*Bgl* II fragment from pNPS427 (17) was used as a probe. Digestion with *Bgl* II and *Bam*HI results in a 3.0-kb band. When this fragment sustains a DSB at the *ARG4* DSB site, two fragments of approximately 1.5 kb result. These 1.5-kb fragments comigrate under our electrophoresis conditions and show up as a single band.

was transferred to a nylon membrane (Zetabind; Cuno) by vacuum in sterile water using a Pharmacia LKB VacuGene XL vacuum transfer apparatus according to the manufacturer's recommendations. DNA was crosslinked to the membrane using a UV crosslinker 1000 (Fisher Scientific). The membrane was then hybridized with a probe made from a gel-purified *Pst* I–*Bgl* II fragment of *ARG4* (see Fig. 1). The fragment was labeled with [α-^{32}P]dCTP using random hexamer primers. Hybridization and wash conditions were as recommended by the manufacturer of Zetabind (Cuno). Membranes were scanned by an AMBIS radioanalytic scanner (described below) and were then exposed to Amersham Hyperfilm for 2 or 3 days at −70°C to obtain an autoradiogram.

Quantification of DSB Frequencies. Membranes were scanned on an AMBIS 4000 radioanalytic scanner (AMBIS Imaging Systems) according to manufacturer's recommendations. The AMBIS directly measures radioactivity on a membrane and responds linearly in a range that includes all measurements taken in these experiments. To quantify the frequency of the DSBs, the amount of radioactivity in the uncut and the DSB bands was determined. Background radioactivity resulting from nonspecific degradation of DNA produced a faint smear starting at the uncut band. It was important to eliminate the background radioactivity from the DSB bands, since these bands were faint. To eliminate background, readings were taken immediately above and below the DSB band. The average of the two background readings was taken to represent the background at the position of the DSB band and was subtracted from the reading taken for the DSB band. The frequency of the DSBs was calculated as the amount of radioactivity in the DSB band divided by the sum of the radioactivity in the DSB and uncut bands.

RESULTS

In diploid yeast meiosis, DSBs at the *ARG4* recombination hot spot can be detected with Southern blot techniques. The DSBs are revealed as a band arising a few hours after the cells have been shifted to a medium that induces meiosis (e.g., Fig. 2*A*). Experiments were conducted in strains carrying the *rad50S* allele. In a *rad50S* strain, DSBs occur and accumulate unprocessed, thus facilitating their detection and quantification. We asked whether *ARG4* DSBs can occur under conditions in which potential pairing sites on the homologue were partially or completely eliminated.

The diploid yeast strain F1007 is an *ARG4* deletion heterozygote. In one parent, a deletion extends from 280 bp 5′ of the *ARG4* DSB site to 5000 bp 3′ of the DSB site (Fig. 1). In this diploid, the possibility for pairing between homologues is eliminated in the immediate vicinity of the *ARG4* DSB site. F1007 was cultured under conditions that induce meiosis and samples were taken at hourly time points throughout the early stages of meiosis. Genomic DNA was prepared and subjected to gel electrophoresis and Southern blot analysis to detect DSBs at the *ARG4* DSB site (7). Fig. 2 shows that DSBs occur in the deletion heterozygote F1007 with a frequency and kinetics approximately equal to the wild-type homozygote (Fig. 2 compare *A* and *B*). Thus, pairing between homologous chromosomes in the immediate vicinity of the *ARG4* DSB site is not necessary for the formation of meiosis-specific DSBs. Consistent with this observation, Nicolas *et al.* (2) showed that a small heterozygous deletion encompassing the *ARG4* DSB site was converted with the wild-type parent acting as the preferred recipient. This is most easily interpreted as meaning that the intact DSB site on the wild-type parent was being acted upon by endonucleases despite the absence of interhomologue pairing possibilities in the immediate vicinity of the DSB site.

The above observations do not rule out the possibility that pairing of chromosome VIII homologues remote from the *ARG4* DSB site is necessary and sufficient for introduction of the DSB. We test this possibility by inducing meiosis in a haploid strain to eliminate the homologue altogether.

Haploid yeast strains defective in the *SIR3* gene express the two silent mating type loci and are proficient to enter meiosis. We inserted a nonrevertible *sir3* mutant allele (19) into a haploid. In haploids, the only possibility for homology-dependent DNA–DNA interaction exists between sister chromatids. F1031, a *sir3* haploid, was analyzed for the presence of meiosis-specific DSBs in the sporulation regimen described above. Fig. 2*C* shows that DSBs occur in the haploid strain. Size standards are on the left side of Fig. 2*A* and (faintly) on the right side of Fig. 2*C*. They demonstrate that the location of the DSB site is approximately the same in haploids and diploids. Experiments using a *sir4* mutation (instead of *sir3*) to allow a haploid strain to enter meiosis produced similar results (data not shown).

To compare better the frequency and timing of DSBs in diploid and haploid meiosis, we quantified the frequency of the DSBs at each time point. In independent experiments, the haploid and diploid strains were subjected to a time course analysis as described above. The number of experiments was six for the diploid and five for the haploid. The frequencies of DSBs were determined from the relative intensities of the bands representing the uncut fragments and the DSB fragments. The results of this analysis for the haploid and diploid strains are graphed in Fig. 3. These data show that the frequency and timing of the DSBs in the haploid and diploid strains are not significantly different (the mean frequencies with standard errors of the mean overlap at 9 out of 10 time points). Small differences in the timing of the DSBs (i.e., less than 2 h) would not have been detected by using our methods.

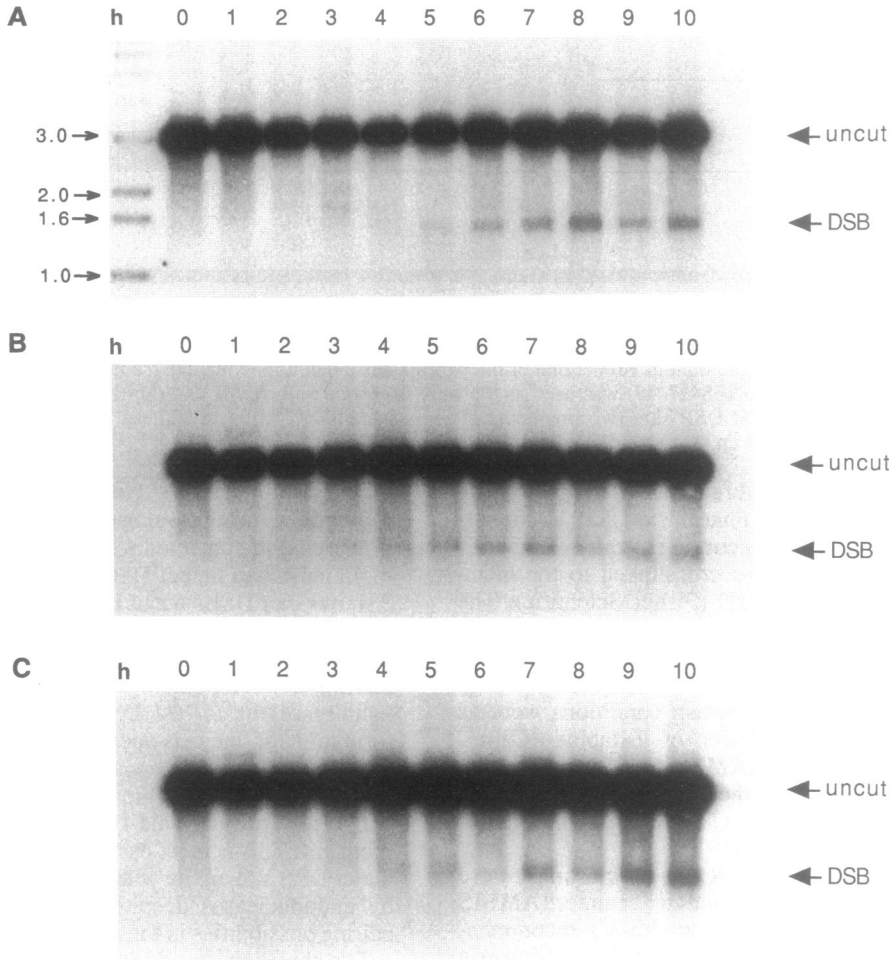

FIG. 2. Autoradiograms from a time course experiment showing the occurrence of meiosis-specific DSBs at the *ARG4* initiation site. The upper more-intense band (uncut) represents the 3.0-kb *Bam*HI–*Bgl* II fragment. The lower less-intense band (DSB) represents the 1.5-kb fragments resulting from the meiosis-specific DSB. (*A*) Wild-type homozygous diploid F1032. (*B*) Deletion heterozygote diploid F1007. (*C*) Wild-type haploid F1031. Size standards are visible on the left of *A* and (faintly) on the right of *C* and are identified in *A* (kb).

Meiosis-specific DSBs at *ARG4* were also seen in an isogenic *RAD50* haploid (data not shown), indicating that homologue-independent DSB formation is not a peculiar property of the *rad50S* allele.

DISCUSSION

In the classical view of meiotic recombination and chromosome pairing (e.g., ref. 23), chromosomes first form SCs and then initiate recombination. Although this view has fallen into disfavor in light of recent observations (refs. 8 and 13; for review, see refs. 10 and 12), recent studies have identified presynaptic homologous associations early in meiosis (11, 14), leading to the possibility that the meiotic cell induces high levels of recombination only after acknowledging the presence of a homologue. The results of our experiments show that the frequency, kinetics, and location of DSBs at the *ARG4* DSB site are the same in haploid and diploid meiosis. These observations demonstrate that interhomologue pairing is not a prerequisite for the occurrence of DSBs, an early event in meiotic recombination.

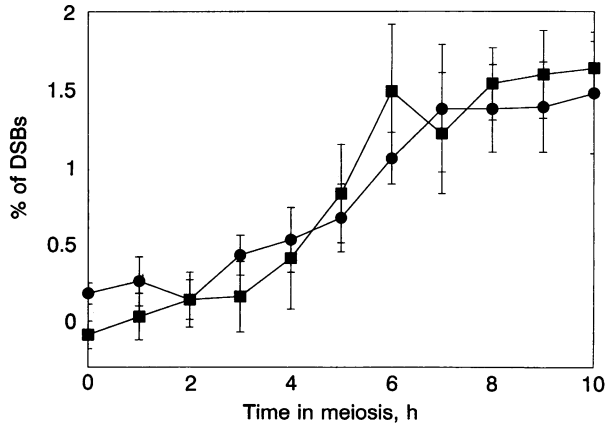

FIG. 3. Graph of the mean frequency (percentage) of DSBs, showing the increase in DSBs over time in the diploid F1032 (●) and the haploid F1031 (■). The error bars represent the SEM. Six experiments were done for the diploid and five were done for the haploid.

Malone (24) reported that haploid meiosis in *rad52* yeast strains leads to the production of inviable spores. Our experiments provide physical evidence for the occurrence of DSBs in haploid meiosis. These DSBs presumably are inefficiently repaired in the absence of *RAD52* function, likely resulting in the observed spore lethality.

Loidl *et al.* (25) showed that extensive SC formation occurs between nonhomologues in yeast haploid meiosis. Thus it is not possible to say that the formation of DSBs that we observed in haploid meiosis is independent of the SC. We note, however, that meiosis-specific DSBs do occur in mutant backgrounds in which normal SCs appear infrequently or not at all (3, 9).

The fate of meiosis-specific DSBs in haploid meiosis was not followed in these experiments. Sun *et al.* (26) noted that sister chromatid recombination on circular and linear minichromosomes is stimulated by the *ARG4* DSB hot spot,

suggesting that both inter- and intrachromosomal recombination can initiate with a DSB. It therefore seems likely that the DSBs occurring in haploid meiosis would be repaired by using the sister chromatid as a template. Indeed, Wagstaff *et al.* (27) showed that intrachromosomal (including sister chromatid) recombination at the *HIS4* locus is significantly more frequent in haploid meiosis than in diploid meiosis. The implication is that, in haploid meiosis where interhomologue recombination is not an option, chromatids with DSBs can and will utilize homology on the sister chromatid for recombinational repair.

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