A Test of the Double-Strand Break Repair Model for Meiotic Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

We tested predictions of the double-strand break repair (DSBR) model for meiotic recombination by examining the segregation patterns of small palindromic insertions, which frequently escape mismatch repair when in heteroduplex DNA. The palindromes flanked a well characterized DSB site at the *ARG4* locus. The "canonical" DSBR model, in which only 5′ ends are degraded and resolution of the four-stranded intermediate is by Holliday junction resolvase, predicts that hDNA will frequently occur on both participating chromatids in a single event. Tetrads reflecting this configuration of hDNA were rare. In addition, a class of tetrads not predicted by the canonical DSBR model was identified. This class represented events that produced hDNA in a "*trans*" configuration, on opposite strands of the same duplex on the two sides of the DSB site. Whereas most classes of convertant tetrads had typical frequencies of associated crossovers, tetrads with *trans* hDNA were parental for flanking markers. Modified versions of the DSBR model, including one that uses a topoisomerase to resolve the canonical DSBR intermediate, are supported by these data.

THE efficiency of chromosomal integration of plasmids in yeast transformation was shown to be increased by prior linearization of the plasmid DNA (ORR-WEAVER *et al.* 1981; ORR-WEAVER and SZOSTAK 1983). The double-strand break (DSB) is repaired using chromosomal DNA as a template during the act of integration. These and other features of plasmid integration parallel meiotic recombination and led to the proposal of the double-strand break repair (DSBR) model for meiotic recombination (RESNICK 1976; SZOSTAK *et al.* 1983). The model predicts that recombination initiates with a DSB (Figure 1A). DSBs clearly play a central role in meiotic recombination. DSBs have been associated with several meiotic recombination hotspots (SUN *et al.* 1989; CAO *et al.* 1990; GOLDWAY *et al.* 1993; OHTA *et al.* 1994; FAN *et al.* 1995). Studies of the timing of the DSBs and mutational analyses support an initiator role for the DSB in recombination (CAO *et al.* 1990; PADMORE *et al.* 1991; BISHOP *et al.* 1992; GOYON and LICHTEN 1993). DSB formation is demonstrably independent of interhomologue interactions (DE MASSEY *et al.* 1994; GILBERTSON and STAHL 1994; FAN *et al.* 1995), also suggesting an initiation role for DSBs.

The DSBR model makes testable predictions about the molecular nature of the recombination intermediates. Following the DSB, the model supposes that 3′ single-stranded tails will be generated by the action of a 5′-3′ exonuclease (Figure 1B). Evidence for such overhangs resulted from studies on populations of cells proceeding through meiosis (SUN *et al.* 1991; BISHOP *et al.* 1992). A mutant allele of the *RAD50* gene, *rad50s*, apparently blocks this processing of the DSBs (ALANI *et al.* 1990; KEENEY and KLECKNER 1995; LIU *et al.* 1995), whereas in other mutant backgrounds (*e.g.*, *dmc1*, *rad51*) processing is abnormally extensive (BISHOP *et al.* 1992; SHINOHARA *et al.* 1992).

The 3′ tails are postulated to invade a homologous duplex (Figure 1C), prime new DNA synthesis and ligate to 5′ ends creating the canonical DSBR model intermediate shown in Figure 1D. The intermediate has two Holliday junctions. The isolation of this intermediate using physical techniques (COLLINS and NEWLON 1994; SCHWACHA and KLECKNER 1994, 1995) provides strong support for the model. According to the canonical model, this intermediate will then be resolved into mature recombinants by a Holliday junction cutting activity. Each Holliday junction can be cut in one of two orientations. Cleaving the two junctions in the same orientation results in a noncrossover product, whereas cleavage in opposite senses results in a crossover product (Figure 1, E and F). Cleavage of the two Holliday junctions at random, independently of each other, would result in half of all gene conversions being associated with crossovers.

The original version of the model (SZOSTAK *et al.* 1983) provided little opportunity for the formation of hDNA. DSB formation was thought to be followed by exonucleolytic degradation of both the 3′- and, to a greater degree, the 5′-ending strands, creating a double-strand gap (DSG). Gene conversion was proposed to occur primarily through repair of the DSG. Indeed, the demonstration of efficient repair of gapped plas-

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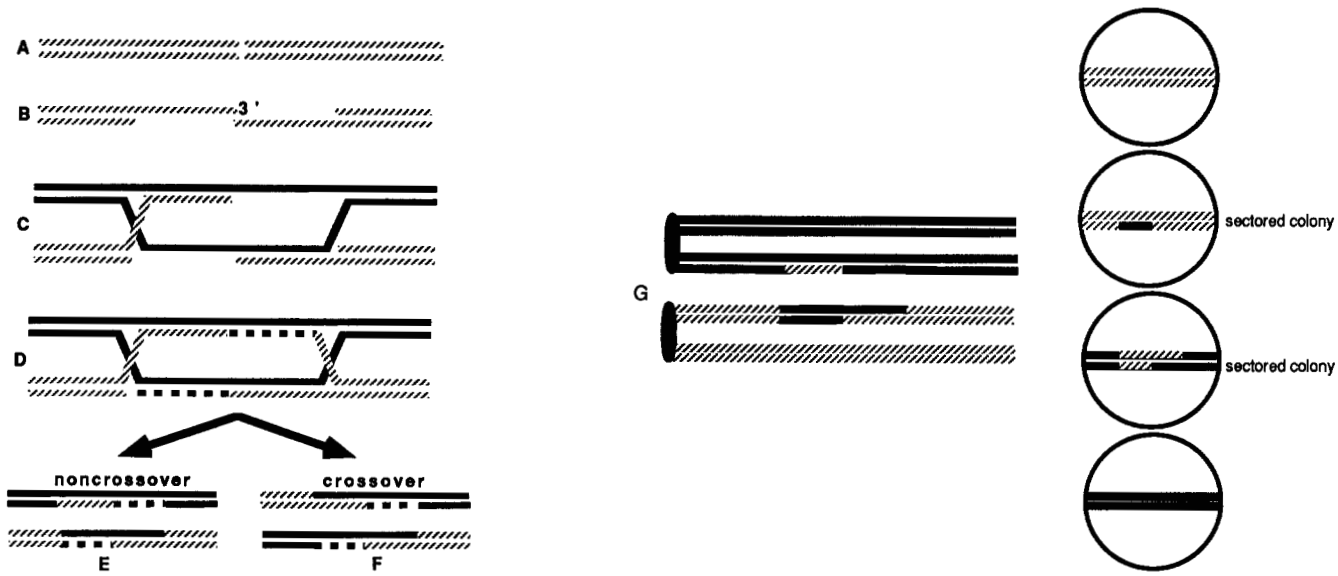

FIGURE 1.—The DSBR model for homologous recombination (A–F). See text for details. Dashed lines indicate newly synthesized DNA. Heteroduplex segregation as predicted by the DSBR model (G). This example shows the products of a simple gene conversion, derived from E. Segregation of the four chromatids into four spores will result in two spores having hDNA on opposite sides of the DSB site. These spores will germinate and give rise to sectored colonies that can be identified as described in MATERIALS AND METHODS.

mids (ORR-WEAVER *et al.* 1981; ORR-WEAVER and SZOSTAK 1983) was a major impetus for the model. The DSG repair pathway for gene conversion would result in mostly full gene conversions. [Full gene conversions are those that result in 6:2 or 2:6 allelic segregation. Half gene conversions, commonly called postmeiotic segregations (PMS), are those that result in a 5:3 or 3:5 segregation.] Existing data from other fungal systems (*i.e.,* Ascobolus and Neurospora) indicated that half gene conversions can be frequent. Later, yeast workers demonstrated that mismatch repair-deficient mutants have increased frequencies of half gene conversion (WILLIAMSON *et al.* 1985). In fact, half conversions increased at the expense of full conversions in these mutants, suggesting that most full conversions result from the repair of mismatches in hDNA. These observations forced a revision of the model to include more hDNA (Figure 1), and it may be that only the 5′ ending single strand is degraded after DSB formation. Leaving the 3′ end intact provides potential for long tracts of hDNA, beginning at the site of the DSB and emanating in both directions.

The DSBR model, as modified to retain fully the 3′-ended strands (absent mismatch correction), predicts that hDNA will be present on both DNA molecules that emerge from a single recombination event (on opposite sides of the initiating DSB, Figure 1G). To gather more information about the distribution of hDNA in a meiotic recombination event, we flanked the *ARG4* recombination initiation site with small palindromic insertions. Half gene conversion of these palindromic insertions revealed the presence and position of hDNA in recombination intermediates and allowed us to test predictions of the model.

MATERIALS AND METHODS

Plasmids: A 3.29-kb *Pst*I fragment containing the *ARG4* gene from pMLC28 (SCHULTES and SZOSTAK 1990) was subcloned into the *Pst*I site of pTZ18U (U.S. Biochemical) to create pLL1. A 0.85-kb *Kpn*I fragment upstream of *ARG4* in pLL1 was deleted to eliminate all *Sal*I sites, creating pLG19. Site-specific mutagenesis was performed on pLG19 using an oligonucleotide with the following sequence: 5′-TGAGTGTCGACTGACA-3′. This mutagenesis created a *Sal*I site within the third and fourth codons of the *ARG4* open reading frame (ORF). The resultant plasmid was named pLG49. A lop (LexA operator) palindrome, 5′-TCGAGTACTGTATGTACATACAGTACTCGA-3′ (NAG *et al.* 1989), was then inserted at the *Sal*I site of pLG49, creating pLG51. This insertion generates the *arg4* frameshift allele, *arg4-1691-lop*. A 2.5-kb *Eco*RI-*Hin*dIII fragment containing the *arg4-1691-lop* allele from pLG51 was subcloned into the *Eco*RI-*Hin*dIII sites of pRS306 (SIKORSKI and HIETER 1989) to create pLG55.

Site-specific mutagenesis (KUNKEL *et al.* 1987) on pLG19 using an oligonucleotide with the sequence 5′-GATTGTCGTCGACAGAGGG-3′ generated a *Sal*I site in place of the *Hpa*I site located −316 bp relative to the beginning of the *ARG4* ORF. This plasmid was named pLG56. A lopC palindrome, 5′-TCGACTAGTCTAAGTACTTAGACTAGTCGA-3′ (NAG *et al.* 1989), was inserted at the *Sal*I site of pLG56, creating pLG57.

pWE118 was constructed by KEN HILLERS (this lab) as follows: the unique *Bgl*II site in pLG19, at the 3′ end of the *ARG4* ORF, was changed to a *Sal*I site by filling in with Klenow and adding an 8-bp *Sal*I linker. The lopC palindrome was then inserted at the new *Sal*I site, creating pWE115. The allele created was called *arg4-BglII-lopC*. A 2.5-kb *Eco*RI-*Hin*dIII fragment from pWE115 was subcloned into the *Eco*RI-*Hin*dIII sites of pRS306, creating pWE118.

A 3.07-kb *Bgl*II fragment upstream of *ARG4* (from −3.9 to

TABLE 1

Yeast strains

Strain	Genotype[a] (or parents)
F1200	*MATa ARG4*
F1201	*MATa ARG4*
F1209	*MATa arg4-1691-lop spo13::URA3-loxP*
F1210	*MATa ARG4::HpaI-lopC DFF::LEU2-loxP trp1-ΔXbaI*
F1216	F1209 × F1210
F1227	*MATa ARG4 DFF::LEU2-loxP spo13::URA3-loxP*
F1233	*MATa arg4-1691-lop;HpaI-lopC*
F1236	F1227 × F1233
F1245	*MATa arg4-1691-lop;BglII-lopC;HpaI-lopC*
F1246	F1227 × F1245
F1247	*MATa arg-4-1691-lop ade1 ade4 lys2*
F1248	*MATa arg4-1691-lop ade1 ade4 his3*
F1249	*MATa arg4-BglII-ClaI ade1 ade4 his3 trp1*
F1250	*MATa arg4-BglII-ClaI ade1 ade4 lys2*

[a] In addition to the listed genotypes, all strains (except F1247–F1250) are also *ura3-52 lys2-HpaI-HindIII leu2-ΔKpnI his3-Δ200 ade2-ΔEcoRV.*

−7.0 kb upstream) from pSPO13-2 (WANG *et al.* 1987) was subcloned into the *Bam*HI site of pTZ18U to create pLG35. A 2.2-kb *Xho*I-*Sal*I fragment containing *LEU2* from YEp13 (BROACH *et al.* 1979) was inserted at the unique *Xho*I site of pLG35 (position of the *Xho*I site relative to the *ARG4* ORF is −4.9 kb) to create pLG39. A sigma element has been identified at the position of this *Xho*I site (JOHNSTON *et al.* 1994). This plasmid was then cut with *Hpa*I. *Bgl*II linkers were added, followed by a *Bgl*II-*Xho*I digestion. This substrate was ligated to a 60-bp *Xho*I-*BamH*I fragment containing a *loxP* recombination site from pBS43 (SAUER 1987), creating pLG42. We call the insert in pLG35 *DFF* (*ARG4 D*istal *F*lanking *F*ragment), and the disruption allele in pLG42 was designated *DFF::LEU2-loxP*. The *loxP* sites in pLG42 and pLG54 (below) were included as part of plasmid constructions for other experiments.

A 1.16-kb *Pst*I-*Eco*RI fragment (+8.37–9.52 kb relative to the start of the *ARG4* ORF) containing the *SPO13* gene from pSPO13-1 (WANG *et al.* 1987) was subcloned into the *Eco*RI-*Pst*I sites of pTZ18U, creating pLG40. An *Hpa*I site within this insert (+8.74 kb relative to the *ARG4* ORF) was changed to a *Bgl*II site by adding *Bgl*II linkers, creating pLG41. *Bgl*II linkers were added to a 1.17-kb *Sma*I fragment containing the *URA3* gene from pJJ242 (JONES and PRAKASH 1990) followed by insertion of this fragment into the *Bgl*II site of pLG41, creating pLG47. A 60-bp *Xho*I-*Bam*HI fragment containing a *loxP* site from pBS43 was inserted into the *Sal*I-*BamH*I sites of pLG47 to create pLG48. pLG48 has two *Hin*dIII sites, one within the *spo13::URA3-loxP* insert, and one at the vector-insert junction. A partial *Hin*dIII digestion of pLG48, followed by Klenow treatment, eliminated the *Hin*dIII site within the insert, creating pLG54.

Yeast strains: All strains were derived from haploid strains F1200 (*MATa his3-Δ200 lys2-ΔHpaI leu2-ΔKpnI ade2-ΔEcoRV ura3-52*) and F1201 (*MATa his3-Δ200 lys2-ΔHpaI leu2-ΔKpnI ade2-ΔEcoRV ura3-52 trp1-ΔXbaI*). F1200 and F1201 are isogenic to strains used in previous work in this lab (FOSS and STAHL 1995). Strains are listed in Table 1. All diploids are isogenic, and various alleles were integrated by one-step (ROTHSTEIN 1983) or two-step transplacement (SCHERER and DAVIS 1979) using a lithium acetate yeast transformation procedure (ITO *et al.* 1983). The *arg4-1691-lop* allele was incorporated into various strains by two-step transplacement with *Bgl*II-digested pLG55. The *ARG4::HpaI-lopC* allele was inserted by one-step transplacement of an *arg4* (typically *arg4-1691-lop*) strain with *Eco*RI-*Hin*dIII-digested pLG57. Arg^+ transformants were screened by the PCR (below) for the presence of the *HpaI-lopC* insertion. The *arg4-BglII-lopC* allele was integrated by two-step transplacement using *Sna*BI-digested pWE118. In the case of F1244, the *ARG4::HpaI-lopC* allele was inserted first, followed by insertion of the *arg4-1691-lop* allele and then the *arg4-BglII-lopC* allele. The *spo13::URA3-loxP* flanking marker allele was inserted by one-step transplacement using *Eco*RI-*Hin*dIII-digested pLG54. The *DFF::LEU2-loxP* flanking marker was inserted by one-step transplacement using *Sac*I-*Pst*I-digested pLG42.

Four unrelated strains (listed in Table 1) were used for allelism tests (described below). These strains are segregants with desired genotypes that were selected from crosses between various laboratory strains.

Genetic analysis: Standard procedures and media were used (TRECO 1992). Diploids were grown for 2 days on YEPD plates as a patch and then replica-plated to a sporulation medium, followed by incubation at 30° for 3 days. Approximately 50% of the cells formed asci with four spores. Tetrads were dissected directly onto thin YEPD plates and incubated for 4 days at 30°. Dissections of F1246 were done on modified YEPD plates with twice the normal concentration of nutrients to increase the size of the spore colonies to facilitate the scoring of sectored colonies in allelism tests. Spore colonies were replica-plated directly to appropriate omission media for phenotypic analysis. For F1246, spore colonies were also replica-plated onto lawns of F1247, F1248, F1249, and F1250, and incubated overnight to allow mating. Diploids were selected by replica-plating onto SD −ade media. Diploid colonies were then replica-plated to a sporulation medium and incubated at 30° for 3 days, followed by replica-plating to SD −arg medium. The sporulation step induced recombination, resulting in the production of prototrophs in heteroallelic diploids. This allowed assignment of allelic genotypes to all segregants of a heteroallelic cross.

Sectored colonies (*i.e.*, colonies with a mixed population of *ARG4* genotypes) identified in this procedure were streaked out on YEPD plates so that individual subclones of each genotype could be obtained for further study. Any colonies for which a clear assignment (sectored *vs.* nonsectored) could not be made in the initial screen were streaked out to produce individual subclones and retested.

Detecting sectors required gentle replica-plating. For F1216 and F1236, where sectors could be detected directly by replica-plating the dissection plate onto SD −arg omission media, reconstruction experiments indicated that we were able to detect sectors with 98% accuracy (data not shown). For F1246, which required a series of replica-platings (see above), it is possible that some members of the 3:5 class (see Table 2) were misidentified as nonconvertant (4:4) tetrads. The fact that no 5:3 tetrads were initially misidentified as 6:2 tetrads argues that few, if any, 3:5 tetrads were misidentified as nonconvertant tetrads.

Selected tetrads were subjected to analysis by PCR to follow the segregation of the *ARG4::HpaI-lopC* allele. PCR was performed individually on all four segregants of the tetrad. In the case of tetrads with a sectored colony, subclones of each sector were analyzed separately. Cells were picked with a plastic pipette tip from fresh colonies (<3 days old) grown on YEPD and were inoculated into 25 μl of a PCR reaction mixture containing 10 U Taq polymerase (Promega), 1× reaction buffer (supplied with the polymerase), 1 mM dNTPs, 10 mM $MgCl_2$, and 20 pmol of each primer. Mineral oil (25 μl) was added after inoculation. Reactions were carried out in a 96-well microtitre dish in a Hybaid OmniGene thermocycler with the following parameters:

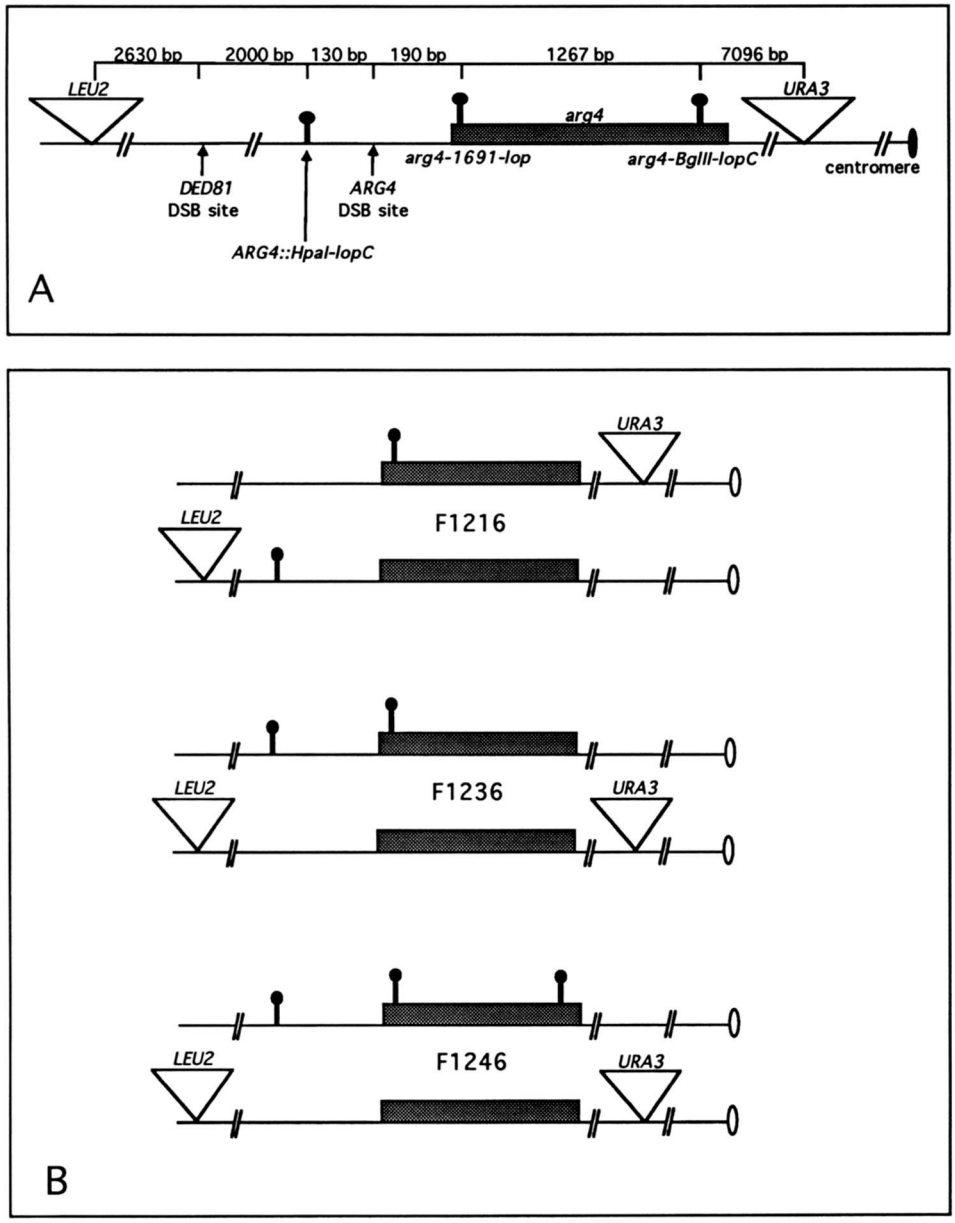

FIGURE 2.—Map of the relevant features of experimental strains. (A) A small region encompassing *ARG4* on chromosome *VIII*. *URA3* is inserted at *SPO13*. *LEU2* is inserted at a sigma element upstream of *ARG4*. (B) The three experimental diploids. See MATERIALS AND METHODS for details.

94° for 10 sec, 52° for 10 sec, 72° for one min; this cycle was repeated 32 times. Ten microliters of the PCR products were subjected to gel electrophoresis on a 3% NuSieve (FMC) agarose gel for 4 hr and visualized by ethidium bromide staining. Oligonucleotides used for PCR were 5′-CAGAGTTCTGTGCTTCGCTG-3′ and 5′-GTATCCACGTTTCAGCGGTAG-3′. These oligos were used to amplify a fragment −506 to −108 bp relative to the *ARG4* ORF, spanning the site of the *Hpa*I-lopC insertion. The size of the fragment was 398 bp without the *Hpa*I-lopC insertion and 424 bp with the insertion. These sizes were resolved by gel electrophoresis.

RESULTS

The present study was designed to test the predictions made by the DSBR model for the configuration of heteroduplex DNA in a meiotic recombination event. Half gene conversion was used as an assay for the presence of heteroduplex. Small palindromic insertions, such as the 30-bp lop palindrome from the LexA operator, have previously been characterized for their effect on PMS and have been exploited for this purpose (NAG *et al.* 1989; DETLOFF *et al.* 1991, 1992; PORTER *et al.* 1993). We constructed diploids (Figure 2) with palindromes flanking the *ARG4* DSB, ~130 bp to the left (*ARG4-HpaI-lopC*) and 190 bp to the right (*arg4-1691-lop*). The *ARG4* DSB site and recombination hotspot has been the subject of numerous studies (ALANI *et al.* 1989; SUN *et al.* 1989, 1991; GOYON and LICHTEN 1993; GILBERTSON and STAHL 1994) establishing its role as an initiator of meiotic recombination at the *ARG4* locus. We confirmed the position of the *ARG4* DSB site in the strains used in this study and showed that it is not dramatically influenced by the presence of the palindromic insertions (data not shown).

The *ARG4::HpaI-lopC* allele has no discernible phe-

TABLE 2

Aberrant segregation of *arg4-1691-lop*

Diploid	Aberrant segregation pattern[a] 6:2	2:6	5:3	3:5	Other	Total tetrads	Total gene conversion
F1216	22	71	50	78	1[b]	2091	10.5%
F1236	9	33	28	29	0	891	11.1%
F1246	11	17	41	27[c]	2[d]	1165	8.3%
Total	42	121	119	134	3	4147	10.0%

[a] Segregation ratios reflect the segregation of the lop palindrome, rather than a phenotype, *i.e.*, the 6:2 ratio means that six of the eight single strands in the pair of homologous chromosomes carried the palindrome.
[b] One aberrant 4:4 segregation pattern was observed.
[c] This class may be slightly underrepresented because of the difficult nature of the allelism assay to detect tetrads of this type (see MATERIALS AND METHODS).
[d] One 1:7 segregation pattern and one 0:8 segregation pattern were observed.

notype and must be scored by a physical assay (see MATERIALS AND METHODS). In most of our analyses, segregation of this allele was determined only for tetrads that were selected on the basis of having had an aberrant segregation of the marker in the *ARG4* coding region.

Flanking markers were included (Figure 2) to allow us to identify gene conversion events that were accompanied by crossovers. The presence of the flanking markers had no effect on the frequency or character of gene conversion of the *ARG4* alleles (data not shown).

Analysis of conversion events: Tetrads (4147) from the three diploids illustrated in Figure 2B were dissected and analyzed. The overall gene conversion frequency of the *arg4-1691-lop* allele was 10%. The class distributions of tetrads recombinant for the *arg4-1691-lop* allele are shown in Table 2. A disparity, shown as an excess of 2:6 types over 6:2 types, is revealed by these data. Disparity can result from differential initiation by the two parental chromosomes. In this case, however, the disparity is more pronounced in tetrads in which correction has occurred. For F1216 and F1236 [F1246 is excluded since the data set is slightly different (χ^2 = 5.5) from those of F1216 and F1236] 5:3/3:5 = 0.73, 6:2/2:6 = 0.32. A contingency chi-square test indicates that these two frequencies are significantly different from each other ($P < 0.001$). That the ratio 5:3/3:5 is closer to parity than is the ratio 6:2/2:6 suggests that the disparity is only partly at the level of initiation or heteroduplex formation. The disparity reflects preferential loss of the palindromic insertion, a result that could be due to excision of the palindrome-containing strand after strand invasion. Depending on which chromosome initiated the recombination event, preferentially removing the palindrome-containing strand from the heteroduplex would result in either a restoration event, or a full gene conversion of the 2:6 type. A repair bias favoring the nonpalindromic strand has been observed previously (DETLOFF *et al.* 1992; PORTER *et al.* 1993).

F1236 is identical to F1216 except for the configuration of the palindromic insertions flanking the *ARG4* DSB site. This allowed us to identify potential marker effects of the palindromes. No statistically significant differences were detected in the distributions of the tetrad classes between the two diploids (Table 2).

In all crosses the frequency of half conversion of the lop palindrome is similar to the frequency seen previously with this marker (NAG *et al.* 1989; DETLOFF *et al.* 1991, 1992; PORTER *et al.* 1993).

Tetrads with an aberrant segregation event at the *arg4-1691-lop* allele were analyzed as described in MATERIALS AND METHODS for the segregation of the *ARG4::HpaI-lopC* allele on the other side of the *ARG4* DSB site. The results of this analysis for three diploids are in Table 3 (F1216 and F1236) and Table 4 (F1246). Fifty-five percent of the tetrads that had an event at *arg4-1691-lop* also had an event at the *ARG4::HpaI-lopC* marker. Nearly all of these events were "coconversion" events; both conversion events involved the same chromatid and, therefore, probably occurred during a single act of recombination. One hundred and forty-six unselected tetrads from F1216 (45) and F1246 (101) were analyzed for the segregation of the *ARG4::HpaI-lopC* allele. The conversion frequency of this allele was 13% (Table 5). Thus, the frequency of coconversion is significantly higher than expected based on the individual frequencies for the two alleles. Frequent coconversion of alleles flanking the *ARG4* DSB site has been described previously (SCHULTES and SZOSTAK 1990). All conceivable combinations of coconversion events were observed.

The DSBR model predicts that heteroduplex DNA will be formed on two emerging chromatids on opposite sides of the DSB site. The frequency at which tetrads reflecting this configuration of hDNA were obtained was low (4/4147), and we cannot exclude the possibility that they arise from two independent recombination events. Detailed description of these rare tetrads is presented in the APPENDIX, along with descriptions of other

TABLE 3

Conversion events in F1216 and F1236

Conversion event							
HpaI-lopC	*1691-lop*	Diploid	Total	C_x	C_o	C_x/C	$P<$
No event	Full	F1216	39	15	24		
		F1236	27	7	16		
		Subtotal	66	22	44	0.33	0.21
Full	Full	F1216	12	10	2		
		F1236	10	5	5		
		Subtotal	22	15	7	0.68	0.01
Half	Full	F1216	40	14	26		
		F1236	9	5	4		
		Subtotal	49	19	30	0.38	0.88
No event	Half	F1216	53	21	32		
		F1236	17	9	8		
		Subtotal	70	30	40	0.42	0.79
Full	Half	F1216	15	10	5		
		F1236	7	4	3		
		Subtotal	22	14	8	0.63	0.04
Half (*trans*)	Half	F1216	20	2	18		
		F1236	9	1	8		
		Subtotal	29	3	26	0.10	0.001
Half (*cis*)	Half	F1216	36	14	22		
		F1236	20	11	9		
		Subtotal	56	25	31	0.45	0.62
		Totals	314	128	186	0.41	

Conversions that are accompanied and unaccompanied by crossovers are enumerated in the columns labeled C_x and C_o, respectively. Note these values include both "associated" and "incidental" exchanges (see text for explanation). C_x/C is the ratio of conversions accompanied by crossovers to total conversion events. $P<$ is the upper limit to the probability that a difference this large or larger between the class value and the average value would arise by chance alone.

unusual tetrads that probably represent the recombinant products of multiple initiation events.

The coconversion tetrads included some that had a half conversion at both *arg4-1691-lop* and *ARG4::HpaI-lopC*. Of these cohalf conversion tetrads, two types were seen (Figure 3). Most had a heteroduplex configuration with newly donated information on one strand of the duplex, the "*cis*" configuration. These events may have been initiated by a DSB site other than the *ARG4* DSB site, with a continuous heteroduplex spanning both markers. The nearest identified meiosis-specific DSB is located in the promoter region of the neighboring *DED81* gene, ~2.1 kb distal to the *ARG4* DSB site (FIGURE 2A, SUN *et al.* 1989; WU and LICHTEN 1994). Distant (*i.e.*, >2 kb) DSB sites normally have a negligible impact on the gene conversion frequency of an allele, but DETLOFF *et al.* (1992) have shown that the use of markers prone to half (instead of full) conversion alters the steepness of a polarity gradient. When such markers are used at the low end of a conversion gradient, they give higher frequencies of aberrant tetrads than is seen with a marker whose heteroduplexes are highly correctable. In effect, the absence of mismatch repair results in abnormally long conversion tracts. Consequently, these *cis* configuration heteroduplexes may have been initiated by the *DED81* DSB site. If so, these tetrads are not informative in a test of the DSBR model.

Thirty-seven cohalf conversion tetrads with a "*trans*" configuration of hDNA were observed (Tables 3 and 4). We assume that these events are initiated at the

TABLE 4

Conversion events in F1246

Conversion event					
HpaI-lopC	*1691-lop*	*BglII-lopC*	Total	C_x	C_o
No event	Full	No event	9	6	3
No event	Full	Full	2	1	1
No event	Full	Half	2	2	0
Full	Full	No event	3	1	2
Full	Full	Full	3	1	2
Full	Full	Half	1	1	0
Half	Full	No event	3	1	2
Half	Full	Full	1	0	1
Half	Full	Half	1	1	0
No event	Half	No event	26	11	15
No event	Half	Full	0	0	0
No event	Half (*cis*)	Half	11	4	7
Full	Half	No event	4	3	1
Full	Half	Full	0	0	0
Full	Half (*cis*)	Half	4	1	3
Half (*trans*)	Half	No event	6	0	6
Half (*cis*)	Half	No event	10	4	6
Half (*trans*)	Half	Full	0	0	0
Half (*cis*)	Half	Full	1	1	0
Half (*trans*)	Half (*cis*)	Half	2	0	2
Half (*cis*)	Half (*cis*)	Half	4	1	3
No event	No event	Full	1	0	1
No event	No event	Half	5	2	3
		Totals	99	41	58

See Table 3 for explanation of abbreviations. The relationship between the two half conversion events in line 9 is *cis*.

ARG4 DSB site and will consider them in more detail in DISCUSSION. In F1246, in which three palindromic insertions were assayed for half conversions, only the pair that flanked the *ARG4* DSB site segregated with the *trans* configuration of hDNA (Table 4). The two alleles within *ARG4, arg4-1691-lop* and *arg4-BglII-lopC,* coconverted frequently, usually with half conversions at both sites but never with the *trans* hDNA configuration. Twenty-one cohalf conversions were seen, all with the *cis* hDNA configuration. These data argue that the *trans* hDNA cohalf conversions involving *arg4-1691-lop* and *ARG4::HpaI-lopC* initiated at the *ARG4* DSB site.

TABLE 5

Gene conversion of *ARG4::HpaI-lopC* in unselected tetrads

	Aberrant segregation pattern				Total tetrads	Total gene conversion
	6:2	2:6	5:3	3:5		
F1216	0	2	0	4	45	13.3%
F1246	2	1	6	4	101	12.9%
Total	2	3	6	8	146	13.0%[a]

[a] Only three of the 19 tetrads that converted *ARG4::HpaI-lopC* coconverted *arg4-1691-lop*.

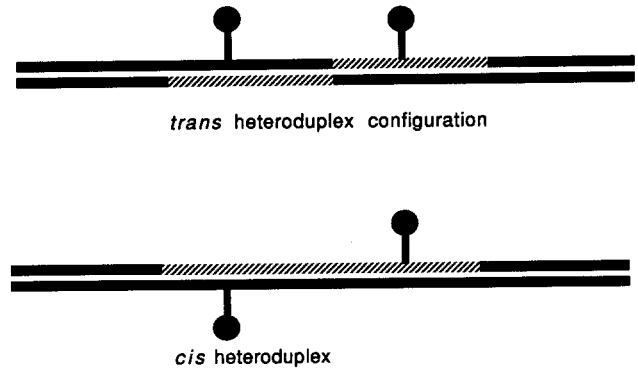

FIGURE 3.—*cis* and *trans* heteroduplexes. The example shown illustrates the palindrome segregation for F1216. *Trans* hDNA contains both palindromes on the same strand. *cis* hDNA puts them on opposite strands. The situation is reversed for F1236.

Other classes of tetrads included many that had a conversion of *arg4-1691-lop* without coconverting *ARG4::HpaI-lopC* (Tables 3 and 4). A similar degree of unidirectionality has been seen previously at the *ARG4* locus (SCHULTES and SZOSTAK 1990) and at the *HIS4* locus (PORTER *et al.* 1993). We will discuss these tetrads in more detail below.

Association of conversion with crossing over: *URA3* and *LEU2* insertions flanked the *ARG4* region (Figure 2). Forty-one percent of all gene conversion tetrads were tetratype for these flanking markers (Tables 3 and 4). For tetrads that show half conversion for either palindromic allele, it is possible to determine whether the accompanying exchange is "associated" with the conversion or "incidental" to it. Associated exchanges have a nonparental linkage of flanking markers on the convertant chromatid (*i.e.,* in the sectored spore colony). Examples of the relationships between the flanking markers and the converted allele for a 5:3 tetrad (the same logic applies to 3:5 tetrads) are illustrated in Figure 4A. This figure also shows that the position of the crossover, proximal *vs.* distal, can be determined. Data from all three diploids indicate that at least 13% of the conversion tetrads that were tetratype for the flanking markers were due to incidental exchanges (Tables 6 and 7). This observed frequency of incidental exchanges can be doubled to estimate the actual frequency of incidental exchanges. Doubling the observed frequency is required since half of all incidental exchanges will, by chance, involve the convertant chromatid and will not appear to be incidental. Thus, we estimate that 26% of the crossovers that occur among tetrads that have undergone a gene conversion at *ARG4* are incidental. The frequency of exchanges that are mechanistically associated with gene conversion at *ARG4,* then, is ~30% [(1−0.26)(0.41)]. This is not significantly different from frequencies seen at this locus in previous work (FOGEL *et al.* 1978).

The same analysis allows one to map the crossover

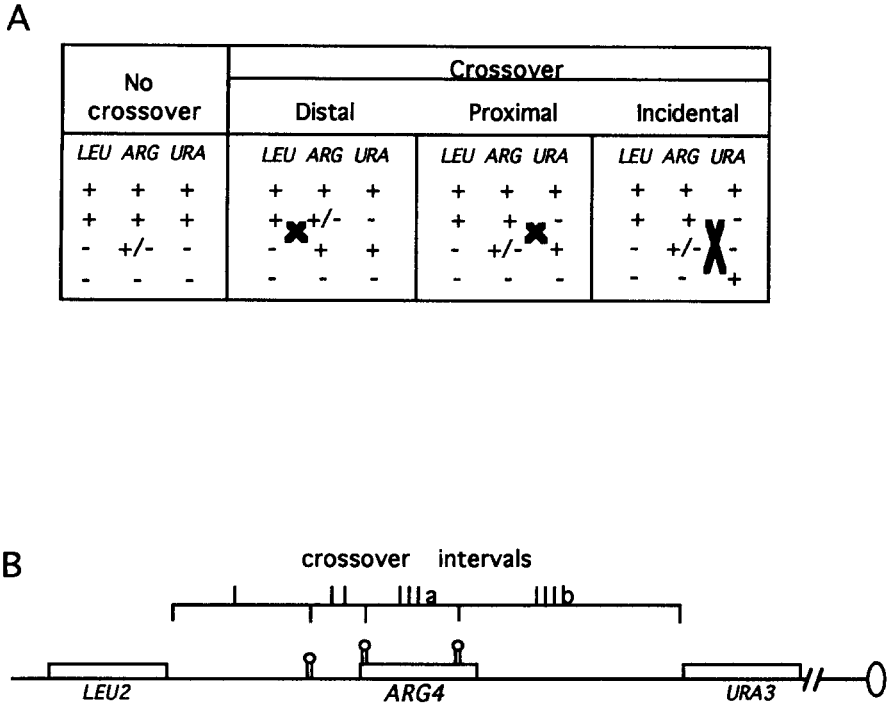

FIGURE 4.—Assay for crossover distribution (modified from FOGEL *et al.* 1978). (A) The segregation of *ARG4* and its flanking markers is shown in the first column for a half conversion unaccompanied by exchange. A conversion that is associated with an exchange in the interval between *ARG4* and *DFF::LEU2-loxP* (the distal interval) will result in the linkages shown in the second column. An associated exchange in the interval between *ARG4* and *spo13::URA3-loxP* (the proximal interval) will result in the linkages shown in the third column. Finally, half of all incidental exchanges will involve the sister of the convertant chromatid. These can be identified by the unique linkages they create, *i.e.*, the convertant chromatid will maintain parental flanking markers, as shown in the last column. B identifies the intervals assayed for crossover distribution.

position (Figure 4A). In its simple form the DSBR model predicts that proximal and distal crossovers will occur at equal frequencies since cleavages of the two Holliday junctions are assumed to be random and independent of each other. In tetrads that had a half conversion event at *arg4-1691-lop,* three intervals (in F1216 and F1236) or four intervals (in F1246) were examined for the placement of the associated exhanges (Figure 4B). Results summarized in Tables 6 and 7 indicate a strong bias in the distribution of crossovers, with most of the associated exchanges occuring in the distal interval (interval I). This is true even when the distal interval is the small region between the palindromic alleles, *i.e.*, tetrads that had converted *arg4-1691-lop* without coconverting *ARG4-HpaI-lopC.* The nature of this bias is unknown. A similar bias, though not as pronounced, was seen at this locus previously (FOGEL *et al.* 1978).

When individual classes of convertant tetrads were examined for crossing over, additional biases were revealed (Table 3). Statistically significant differences in the crossover frequencies were seen in two classes. In the class of tetrads in which cohalf conversion with the *trans* configuration occurred, only 3/37 tetrads had an exchange of flanking markers (Tables 3 and 4). Two of these were obviously incidental exchanges, and the third is likely to have been so. The paucity of crossovers in this class is in striking contrast with the relative abundance of crossovers, 43%, in the class of tetrads with the *cis* cohalf conversion configuration. The decrease in the frequency of crossovers that accompanied *trans* cohalf conversion tetrads is statistically significant ($P < 1.6 \times 10^{-5}$) when compared with the frequency of crossovers in all classes of conversion tetrads combined.

Full gene conversion events at the *ARG4::HpaI-lopC* site are enriched for crossovers (Table 3). The fraction of full conversions that are accompanied by crossing over is ~66%, whether the *arg4-1691-lop* allele is fully or half converted. When the two classes that had a full conversion of *ARG4::HpaI-lopC* are combined, the fre-

TABLE 6

Distributions of crossovers in tetrads with half gene conversion events

	Crossover distribution			
	Crossover interval			
Diploid	I	II	III	Incidental exchanges
F1216	21	15	6	5[a]
F1236	12	3	4	6[b]

[a] All of these incidental exchanges were in interval I.
[b] Five of these incidental exchanges were in interval I, one was in interval III.

TABLE 7

Distributions of crossovers in F1246 tetrads with half gene conversion events

	Crossover distribution				
	Crossover interval				
Allele(s) converted	I	II	III-A	III-B	Incidental exchanges
1691-lop	10	3	1	2	2[a]
BglII-lopC	0	0	0	2	0
Both	4	1	0	1	0

[a] Both of these exchanges were in interval I.

quency of crossovers is significantly different from the populational average ($P < 0.0005$).

Unidirectionality of gene conversion: PORTER *et al.* (1993) observed that gene conversion events frequently extend in only one direction from an initiation site at the *HIS4* locus. Studies examining the full conversion behavior of alleles closely flanking the *ARG4* DSB site (SCHULTES and SZOSTAK 1990) showed that an allele located ~200 bp to the left of the DSB site coconverted with an allele in the *ARG4* gene 64% of the time. Our data confirm this unidirectionality using markers that experience both full and half gene conversions and are closer to the initiation site under study. Forty-five percent of the gene conversion events at *arg4-1691-lop* failed to include the marker on the other side of the initiation site, *ARG4::HpaI-lopC* (Tables 3 and 4). Strikingly, even when the *arg4-1691-lop* conversion tract extended 1.4 kb to include *arg4-BglII-lopC, ARG4::HpaI-lopC* was coconverted only 53% of the time (Table 4), even though it lies only 130 bp to the other side of the DSB site. This phenomenom is not limited to the *arg4-1691-lop* allele. When unselected tetrads were surveyed for aberrant segregation of *ARG4::HpaI-lopC,* only three of 19 conversion events coconverted *arg4-1691-lop* (Table 5).

DISCUSSION

The DSBR model makes specific predictions about the frequency and position of heteroduplex DNA in the recombination intermediate. We tested those predictions using genetic markers that result in a high frequency of half gene conversions and thereby identify heteroduplex DNA.

Figure 1G illustrates how the DSBR model predicts that hDNA will be present on both DNA molecules that emerge from a single recombination event on opposite sides of the site of the initiating DSB. The example in Figure 1G shows a simple gene conversion event without an associated crossover. This particular prediction was unfulfilled. We saw only 4/4147 events with two half gene conversion events segregating separately at *ARG4:HpaI-lopC* and *arg4-1691-lop.* All four of these were associated with exchange of flanking markers and will be discussed below. Previous work (PORTER *et al.* 1993) also failed to find support for hDNA on two separate chromosomes. In addition, existing data revealing a general lack of a particular class of half gene conversion events known as aberrant 5:3 gene conversion (FOGEL *et al.* 1978) constrain the DSBR model with respect to this particular prediction (aberrant 5:3 gene conversion is defined and discussed in FOGEL *et al.* 1978).

The most striking feature of the data comes from a class of tetrads in which heteroduplex DNA spanned markers on both sides of the *ARG4* DSB site with the donated information on the two sides on opposite strands of the same duplex, the *trans* configuration (see Figure 3). This configuration of hDNA is not expected from the canonical DSBR model. Furthermore, unlike all other classes of convertant tetrads, this interesting class is mostly parental for flanking markers (Tables 3 and 4). In fact, of the three tetrads that were tetratype for flanking markers among the 37 tetrads in this class (Table 3), two were demonstrably due to incidental exchanges, *i.e.,* involved chromatids other than the chromatid with the hDNA, and the other may have been incidental as well. It is reasonable to suppose, therefore, that intermediates that are resolved to give the *trans* configuration of hDNA are never associated with a reciprocal exchange.

In interpreting the data we wish to limit our analysis to only those gene conversion events generated by DSBs at the *ARG4* initiation site. The presence of other DSB sites in the region, most notably the *DED81* DSB site (Figure 2A), makes this analysis inherently difficult. Attempts to eliminate the activity of the *ARG4* DSB site with small deletions were unsuccessful. While we cannot rule out the influence of nearby DSB sites on gene conversion of the alleles in our crosses, we have the following reasons for speculating that few gene conversion events are initiated at the *DED81* (or other) DSB site.

1. Any conversion events that included a full gene conversion would probably have been constrained by the mismatch-repair dependent conversion gradient (DETLOFF *et al.* 1992; ALANI *et al.* 1994) to have initiated at a nearby (*i.e., ARG4*) DSB site rather than a distant one.

2. Any conversion events that lacked a full conversion may have been initiated at a distant (*i.e., DED81*) DSB site, but, in the case of conversions *of arg4-1691-lop,* would have coconverted *ARG4-HpaI-lopC.*

3. In the context of the DSBR model it is difficult to imagine how *trans* cohalf conversions could have initiated at a site other than the *ARG4* DSB site, assuming they represent single initiations.

These three points cover all of the conversion classes except for the *cis* cohalf conversions. These may have been initiated at *DED81.* In any case the uncertainty about the origin of the *cis* cohalf conversions does not impact the major conclusions in the following discussion.

As a test of the DSBR model, the most informative tetrads are those in which half gene conversions occurred on both sides of the DSB site. Of the two classes of these cohalf conversion tetrads, we believe that the *trans* cohalf conversion events initiated at the *ARG4* DSB site. In a cross with three palindromic insertions segregating (F1246, Table 4), we note that the *trans* configuration of hDNA was seen only for the palindrome markers that flanked a DSB site. Although many cohalf conversion events involving two alleles within the *ARG4* coding region were observed, these never had the *trans* configuration of hDNA (Table 4). PORTER *et al.* (1993)

performed a similar study of coconversion using palindromic insertion alleles that flanked the *HIS4* recombination hotspot. This study, which lacked flanking markers, also revealed the presence of tetrads with the *trans* configuration of hDNA. When the *HIS4* recombination hotspot was inactivated by a deletion of a participating Rap1p binding site, this class of tetrads disappeared. The *cis* cohalf conversions in the *HIS4* study, on the other hand, were not affected by the deletion of the hotspot, implying that these events initiated elsewhere.

Basic features of the DSBR model have been strongly supported by recent experiments that examined the recombination intermediates using physical techniques (SCHWACHA and KLECKNER 1995). This work has provided compelling evidence for the existence of the DSBR intermediate shown in Figure 1D. This, combined with previous work demonstrating the existence of earlier DSBR intermediates (SUN *et al.* 1989, 1991; ALANI *et al.* 1990; CAO *et al.* 1990; BISHOP *et al.* 1992), motivates us to interpret our results in the context of the canonical DSBR model, while acknowledging that some of our results are consistent with models invoking single-strand gap intermediates (PORTER *et al.* 1993).

There are, in principle, three ways of resolving the structure shown in Figure 1D to give products of the type that we and others (MCGILL *et al.* 1989; PORTER *et al.* 1993) have observed. Resolution can involve the cleavage of two, one, or no Holliday junctions. In the first case, Holliday junction cleavage must be preceded by branch migration of one of the two Holliday junctions to the approximate position of the initiation site. This will establish the *trans* hDNA configuration. Branch migration is followed by cleavage of the two Holliday junctions. Cleavage must occur in the same orientation, either both vertically or both horizontally (depending upon which Holliday junction migrates), to avoid crossing over and to generate the *trans* hDNA configuration. These two constraints (specificities in Holliday junction migration and cleavage) make this model unattractive.

A resolution involving cleavage of one Holliday junction is shown in Figure 5, B and C. In this version, one Holliday junction is cleaved, leaving behind a pair of unsealed single-strand nicks. The remaining Holliday junction then slides, perhaps without direction, but eventually reaching the nicks where it is passively resolved. The products will be parental for flanking markers, regardless of the orientation of the initial Holliday junction cleavage. One of the products will have the *trans* hDNA configuration.

Finally, resolution of the canonical DSBR intermediate can be achieved without Holliday junction cleavages as previously proposed (NASMYTH 1982; THALER *et al.* 1987; HASTINGS 1988; MCGILL *et al.* 1989). In this model, shown in Figure 5, D and E, Holliday junctions converge as the plectonemic joints are resolved by a type I topoisomerase. Again, noncrossover products are formed, one of which has the *trans* hDNA configuration. The data do not allow us to distinguish between the two attractive models shown in Figure 5.

Mutant alleles of both Top1, a type I topoisomerase, and Top2, a type II topoisomerase, increase mitotic recombination in rDNA sequences (CHRISTMAN *et al.* 1988). *TOP2* is required at the first meiotic division for the segregation of chromosomes that have recombined, but a role in the recombination process has not been demonstrated (ROSE *et al.* 1990; ROSE and HOLM 1993). A null mutation in the *TOP3* gene, which encodes a type I topoisomerase homologous to TopA from *Escherichia coli,* results in a block in meiosis (WALLIS *et al.* 1989). The nature of this block is similarly unclear. A mutation in a fourth putative topoisomerase gene, *HPR1,* has no obvious effect on meiotic recombination (AGUILERA and KLEIN 1989).

Trans cohalf conversion events resemble the products of the mating type switching reaction (MCGILL *et al.* 1989). The topoisomerase resolution version of the DSBR model was first proposed, in fact, as a mechanism for mating type switching (NASMYTH 1982). Screening existing topoisomerase mutants for effects on mating type switching could uncover a role for these enzymes in this model recombination reaction.

Trans cohalf conversion tetrads, albeit highly informative, were obtained at a low frequency (0.9%). We wondered how representative these events were of general meiotic recombination. The expected frequency of such tetrads can be calculated from individual frequencies of several events, assuming all of the following events are independent: half conversion of the *arg4-1691-lop* allele (0.06, Tables 3 and 4), coconversion of *ARG4::HpaI-lopC* (0.55, Tables 3 and 4), half conversion of *ARG4::HpaI-lopC* (0.74, Tables 3 and 4), lack of associated exchange (0.70), and initiation at the *ARG4* DSB site rather than another site (0.82). The product of these individual probablities is 0.014 and predicts 58 tetrads of this type. Thirty-seven were seen (Tables 3 and 4). A number of uncertainties enter into the prediction. For example, it is uncertain how many of the observed events were initiated at the *ARG4* DSB site. The frequency of lack of associated exchanges is a corrected value based on an estimate of the frequency of incidental exchanges. Also, independence of correction of hDNA at the two alleles may be an erroneous assumption. In view of these uncertainties, the observed and predicted numbers are close enough to suggest that the mechanism that produced the *trans* co-PMS recombinants is responsible for all gene conversions that are unassociated with exchange.

Our results provide little information on the pathway that gives rise to gene conversions with associated exchange. The original DSBR model (SZOSTAK *et al.* 1983) proposes that crossovers are produced when the two Holliday junctions are cleaved in opposite orientations. The products predicted by this reaction (Figure 1F)

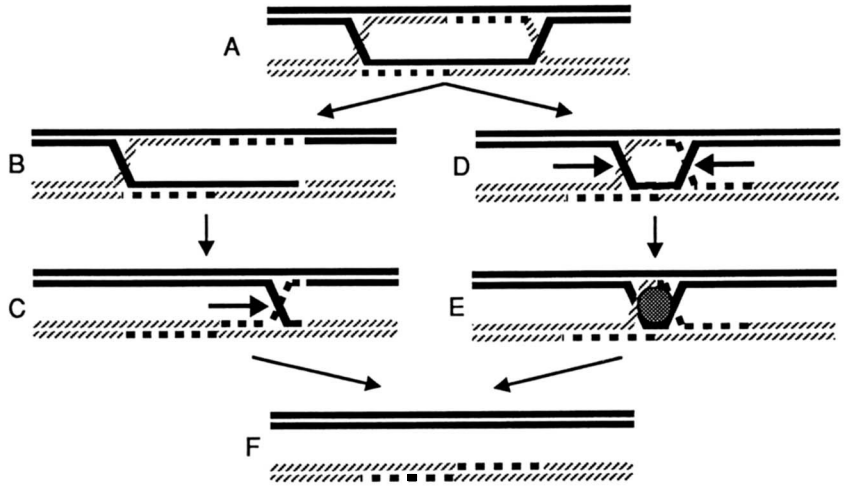

FIGURE 5.—Two alternative DSBR resolutions. The intermediate (A) is the same as in the DSBR model (Figure 1D). Dashed lines indicate newly synthesized DNA. This intermediate may be resolved by cutting one Holliday junction (B and C) or by cutting neither Holliday junction (D and E). In the former case, junction cleavage leaves single-strand nicks on both molecules (B). The remaining Holliday junction is free to move, and eventually slides to the site of the nicks, resulting in resolution to give (after ligation) the products shown (F). In the latter case, the Holliday junctions converge (D), presumably with the assistance of a topoisomerase to relieve the supercoiling that will occur when they do. A topoisomerase acts on the final intertwinings (E) to resolve the two molecules, giving the products shown (F).

were rare in this work and in previous work (PORTER *et al.* 1993). Crossovers are associated with gene conversion about one-third of the time (discussed in FOSS *et al.* 1993; our results). Thus, there should be one tetrad of the type predicted by the DSBR model's crossover pathway for every two *trans* cohalf conversion tetrads. This line of reasoning predicts 18 such tetrads among the unselected population from the three diploids, yet only four were observed.

The lack of tetrads supporting the DSBR model's crossover pathway may be related to the elevated frequency of crossovers among tetrads that have had a full gene conversion at *ARG4::HpaI-lopC* or at both palindromic alleles. For F1216 and F1236 combined, this frequency is 0.66 for tetrads with a full gene conversion at *ARG4::HpaI-lopC* (Table 3). This is a statistically significant increase over the populational average ($P < 0.0005$). These data suggest that heteroduplex correction is associated with crossing over, or vice versa. In either case, this relationship may result in fewer tetrads of the type predicted by the DSBR model's crossover pathway. It is intriguing that this relationship between elevated crossover frequencies and mismatch repair is seen only for the *ARG4::HpaI-lopC* allele. Most crossovers occur on the side of the DSB on which *ARG4::HpaI-lopC* is located (Tables 6 and 7). In fact, this is true specifically for the class of tetrads that had a full conversion of *ARG4::HpaI-lopC* and a half conversion of *arg4-1691-lop*. Of the 17 associated exchanges in this class, 16 occurred on the side on which *ARG4::HpaI-lopC* is located. The bias is lost in the class of tetrads that had a half conversion of *ARG4::HpaI-lopC* and a full conversion of *arg4-1691-lop*. In this class, 10/21 crossovers occur on the side of the DSB on which *arg4-1691-lop* is located. Thus, there is a tendency for crossovers on one side of the DSB site to be correlated with full conversions.

One way to think of a relationship between crossing over and full gene conversion in the context of the DSBR model is depicted in Figure 6. We suppose that Holliday junction cleavage is frequently asynchronous and occurs with sequence and strand specificity. A Holliday junction resolvase from *E. coli* has been shown to possess the latter qualities (SHAH *et al.* 1994). In the example shown, the Holliday junction on the right is frequently the first to be cleaved. This bias sets the stage for the positive correlation between full conversions of the *ARG4::HpaI-lopC* allele and crossing over, and the tendency for crossovers to occur on the left of the initiation site. The strand specificity of the initial Holliday junction cleavage is also an important feature of this version of the DSBR model. We propose that the single-strand nicks produced in the act of Holliday junction cleavage can provoke a second round of excision repair, with the discontinuities directing the removal of strands on one or both duplexes. In the example shown, if the nicked strand on the upper participant is removed (Figure 6C) and replaced by repair synthesis (Figure 6D), the marker of the right side of the DSB site (*i.e., arg4-1691-lop*) will experience a half conversion, while the marker on the left (*i.e., ARG4-HpaI-lopC*) will be fully converted (Figure 6, E and F). The second Holliday junction will then be cleaved in the opposite orientation of the first Holliday junction cleavage, resulting in a crossover.

The biases in Holliday junction cleavage (asynchrony with cleavage occurring first on the right and strand specificity) would lead to the biases reflected in the data: crossovers are positively associated with full conversions, but only for conversions of the *ARG4-HpaI-lopC* allele; crossovers tend to occur on the left of the DSB site.

Figure 6 shows strand excision for only one duplex. It is possible that strand excision can occur on both duplexes. If strand excision occurs on the bottom duplex, the marker on the right side (*arg4-1691-lop*) would be restored rather than converted, and these events would not have been scored in our system that selected tetrads on the basis of having had a conversion event of that allele. If strand excision occurs on neither par-

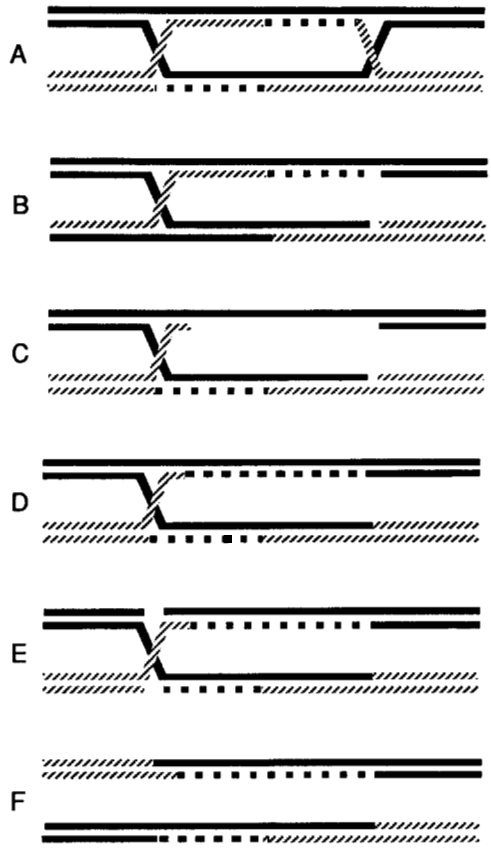

FIGURE 6.—Mismatch repair associated with crossing over. (A) The canonical DSBR model intermediate. (B) Holliday junction resolution occurs asynchronously, with sequence and strand specificity, with the first cleavage occuring on the right as indicated. (C) The residual nicks (after cleavage of the Holliday junction) can direct mismatch repair functions to remove the discontinuous strand. In the example shown, strand excision occurs only on the upper participant to produce a large single-strand gap. (D) Repair DNA synthesis fills in the gap. (E) The orientation of cleavage of the remaining Holliday junction is opposite to that of the first Holliday junction cleavage. (F) After ligation, crossover products are formed that have a full gene conversion on the left of the DSB site, and a half gene conversion on the right. See text for further discussion.

ent, the crossover products would both retain hDNA and would be as predicted by the canonical DSBR model (Figure 1). We saw only four tetrads of this type, whereas we expected 18 (see above). Four is approximately the number of events that we would predict if the strand excision proposed in Figure 6 has a probability of ~0.5 for each parent since 0.25 recombination events would escape strand excision entirely on both duplexes ($0.25 \times 18 = 4.5$).

A second round of mismatch repair stimulated by Holliday junction cleavage (in the context of the DSBR model) has been proposed by ALANI *et al.* (1994) to account for the lack of evidence for symmetric heteroduplex DNA among the products of meiotic gene conversion in yeast.

In addition to the interesting relationship between *trans* hDNA and crossing over revealed by this study, the data suggest frequent asymmetry in individual recombination reactions. Roughly half of all tetrads that had experienced a gene conversion event to the right of the *ARG4* DSB site failed to coconvert the allele to the left of the DSB site (Table 4), even though that allele is only 130 bp from the initiation site. This unidirectionality is illustrated most dramatically by tetrad data from F1246, in which many gene conversion events spanned 1.4 kb to the right of the *ARG4* DSB site, coconverting both alleles in the *ARG4* coding region, without extending another 130 bp to the left. If the DSBR event were perfectly symmetrical from the point of initiation, all conversions that involved the *arg4-1691-lop* allele, and certainly the *arg4-BglII-lopC* allele, should have coconverted the *ARG4::HpaI-lopC* allele since it is closest to the DSB site. Furthermore, this unidirectionality apparently operates in both directions from the *ARG4* DSB site; among the small number of unselected tetrads summarized in Table 5, 16/19 that converted the *ARG4::HpaI-lopC* allele failed to coconvert the *arg4-1691-lop* allele.

Previous studies examining the products of DSB-induced gene conversion in mitotic cells revealed similar asymmetry (SWEETSER *et al.* 1994). In these experiments, in which recombination between *URA3* alleles was induced by HO cleavage of a HO nuclease recognition sequence, unidirectionality was frequently observed with a resolution of 20–30 bp.

Which step in the event is asymmetrical? The first step at which asymmetry can enter is at the point of exonucleolytic action on the double-strand ends. PORTER *et al.* (1993) proposed that roughly half the time only one end is acted upon by a nuclease to generate a single-stranded tail. In the framework of the DSBR model the processed end can then invade a homologue. The other end, remaining primarily double-stranded, may be reluctant or unable to invade. Instead, it may itself be invaded by the exposed single-stranded loop being thrown off by the donor molecule. When the mostly unresected end finally does enter the event, its pairing interaction may be limited to small lengths (*e.g.*, <130 bp in the present study), just enough to stabilize the intermediate. The intermediate would have the same physical properties as the canonical DSBR model intermediate, but the two Holliday junctions would be placed asymmetrically around the initiation site. Previous work has estimated the average size of single-stranded tails at 600 nucleotides (SUN *et al.* 1991; BISHOP *et al.* 1992).

An alternative explanation allows for symmetric resection of the two ends. In this hypothesis, illustrated in Figure 7, the DSB is processed symmetrically to produce long 3′ overhangs (Figure 7A) as proposed by the canonical DSBR model. Instead of invading a single template, the two ends of the DSB often invade separate templates (Figure 7B). In the example shown, one end

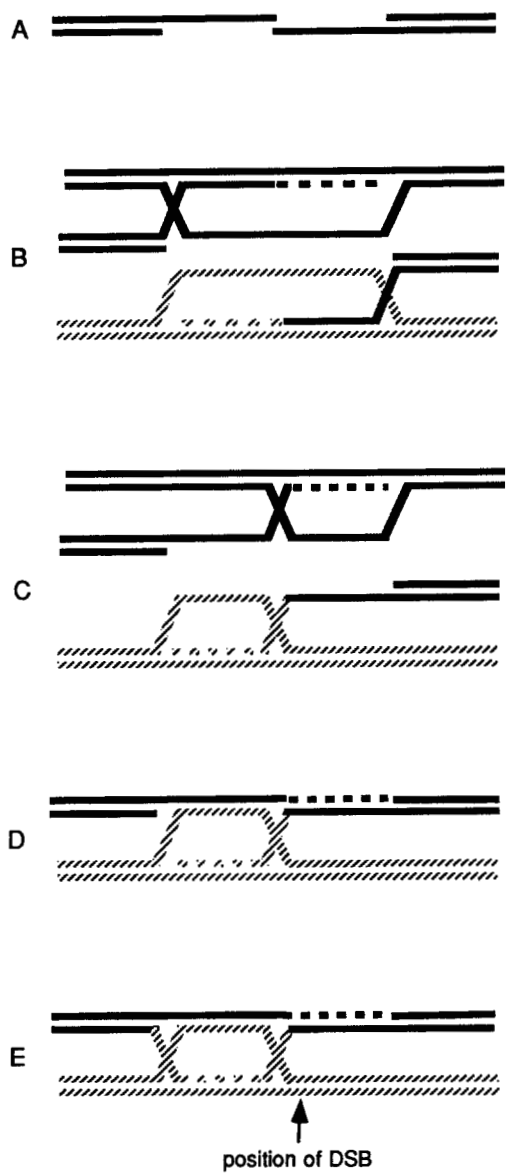

FIGURE 7.—The template choice model for DSBR to account for unidirectionality of gene conversion. See text for details.

invades the sister chromatid (black), while the other end invades the homologue (hatched), and DNA synthesis (dashed line) extends the 3′ ends. Next, Holliday junction migration moves in the same direction as the new DNA synthesis (Figure 7C) such that there is no hDNA at this intermediate step. These single-ended invasions are unstable, and a stable intermediate is formed only when the two invading ends have settled onto the same template and become joined to each other. In the example shown, the end that had invaded the sister chromatid withdraws from that interaction and enters the reaction with the homologue (Figure 7D), forming hDNA and a structure that, after ligation (Figure 7E), contains two Holliday junctions and resembles the canonical DSBR intermediate, except that now the Holliday junctions and the intervening hDNA are all located to one side of the position of the initiating DSB. Resolution of this intermediate may proceed by cleavage of both Holliday junctions as proposed for the DSBR model (Figure 1) or by either of the pathways shown in Figure 5.

Conclusions: Our observations rule out the noncrossover pathway of the canonical DSBR model and suggest alternative mechanisms for resolution of the DSBR intermediate to give crossovers or noncrossovers. The pathway that results in noncrossovers may involve cleavage of a single Holliday junction or the use of a topoisomerase instead of a Holliday junction resolvase to complete the recombination reaction. The data support the crossover pathway of the DSBR model only if it is modified to include a second round of mismatch repair stimulated by Holliday junction cleavage.

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APPENDIX

Eleven tetrads from F1216, two from F1236, and nine from F1246 had complex segregation patterns of the palindromic alleles and/or the flanking markers. These tetrads are shown in detail in Figures 8 and 9. Included in Figure 8 are four tetrads (from F1216) that showed the type of segregation of hDNA pre-

dicted by the DSBR model. These four tetrads all had a crossover (one of them had a double crossover).

Most of the complex patterns of segregation, perhaps even the four discussed above, are likely to be the products of two or more independent recombination events and will not be considered further.

The flanking markers, *spo13::URA3-loxP* and *DFF::LEU2-loxP,* and the *TRP1* allele ocassionally experienced gene conversion. Data on these events are summarized in Table 8. Most of these conversions were independent events, although the *DFF::LEU2-loxP* locus may have coconverted with *arg4-1691-lop* at least four times.

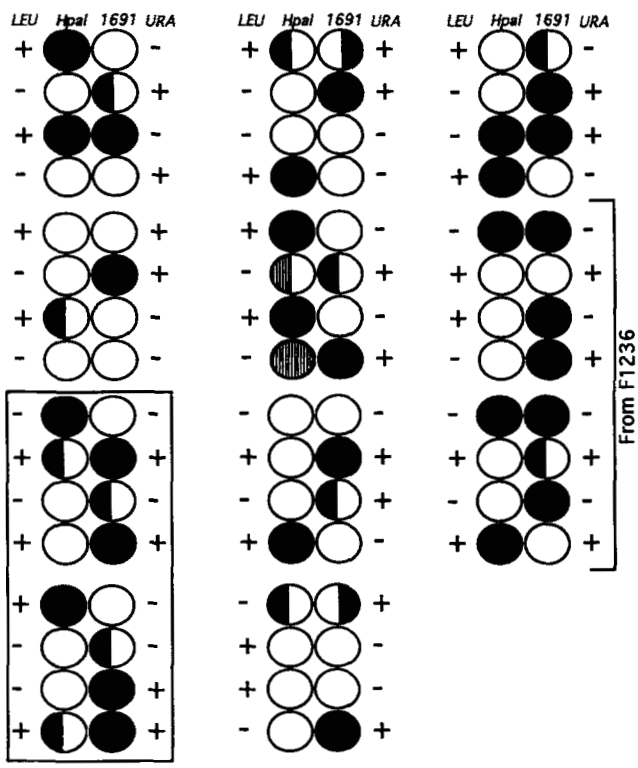

FIGURE 8.—Complex tetrads from F1216 and F1236. The format is similar to that used by PORTER *et al.* (1993). Shading indicates the presence of the palindrome in that segregant. Full shading means that the palindrome was homoduplex; half shading means that the palindrome was heteroduplex. All but the last two in the right column are from F1216. The boxed tetrads displayed segregation patterns that are predicted by the canonical DSBR model. Of the two in the box, the upper one was seen once, the lower one was seen three times. All other tetrad types shown were seen only once. The hatched filling shown in the second tetrad in the second column represents a small (<100 bp) deletion that was created and segregated during a meiosis.

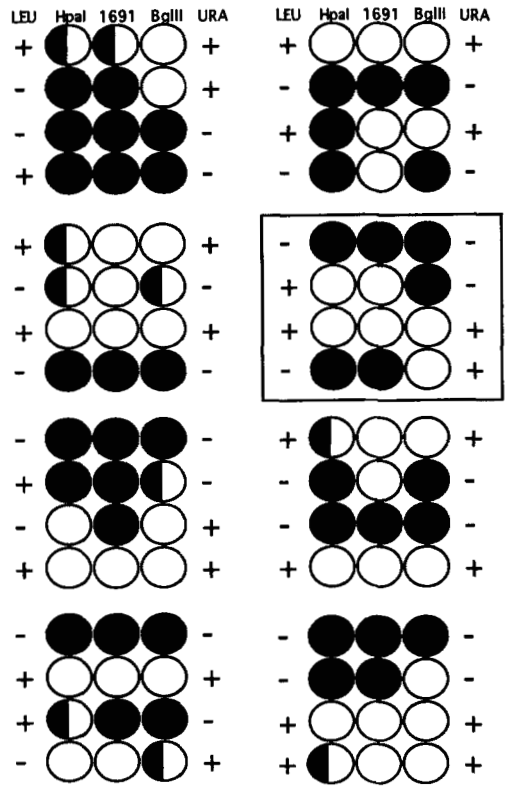

FIGURE 9.—Complex tetrads from F1246. See Figure 8 legend for an explanation of the format. All tetrad types were seen only once, except the boxed tetrad type, which was seen twice.

TABLE 8

Gene conversions of markers other than *ARG4*

Diploid	Segregation	Marker		
		TRP1	*spo13::URA3*	*DFF::LEU2*
F1216	6:2	5	4	19[a]
	2:6	7	5	11[b]
	0:8	0	0	3
F1236	6:2	4	1	1
	2:6	0	1	3
	0:8	0	0	1
F1246	6:2	4	2	3
	2:6	6	3	7

[a] Six of these coconverted *arg4-1691-lop.* Four of the six were apparent single event coconversions since they went in the same direction and included the intervening *ARG4::HpaI-lopC* marker.

[b] One of these coconverted *arg4-1691-lop.*