Reduced Mismatch Repair of Heteroduplexes Reveals “Non”-interfering Crossing Over in Wild-Type *Saccharomyces cerevisiae*

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ABSTRACT

Using small palindromes to monitor meiotic double-strand-break-repair (DSBr) events, we demonstrate that two distinct classes of crossovers occur during meiosis in wild-type yeast. We found that crossovers accompanying 5:3 segregation of a palindrome show no conventional (*i.e.*, positive) interference, while crossovers with 6:2 or normal 4:4 segregation for the same palindrome, in the same cross, do manifest interference. Our observations support the concept of a “non”-interference class and an interference class of meiotic double-strand-break-repair events, each with its own rules for mismatch repair of heteroduplexes. We further show that deletion of *MSH4* reduces crossover tetrads with 6:2 or normal 4:4 segregation more than it does those with 5:3 segregation, consistent with Msh4p specifically promoting formation of crossovers in the interference class. Additionally, we present evidence that an *ndj1* mutation causes a shift of noncrossovers to crossovers specifically within the “non”-interference class of DSBr events. We use these and other data in support of a model in which meiotic recombination occurs in two phases—one specializing in homolog pairing, the other in disjunction—and each producing both noncrossovers and crossovers.

IN yeast, deletion of the meiosis-specific gene *MSH4*, which, despite its name, is said to have no involvement in mismatch repair (ROSS-MACDONALD and ROEDER 1994), usually leaves residual crossovers, and these crossovers have reduced interference (NOVAK *et al.* 2001). In *Caenorhabditis elegans*, however, which is characterized by strong crossover interference as well as by *cis*-acting “pairing centers” that promote synapsis of homologous chromosomes (DERNBURG *et al.* 1998; MACQUEEN *et al.* 2005; PHILLIPS and DERNBURG 2006), deletion of *him-14*, a homolog of *MSH4*, eliminates essentially all crossing over while apparently leaving intact the ability to repair meiotic double-strand breaks (ZALEVSKY *et al.* 1999). On the basis of these data, ZALEVSKY *et al.* (1999) suggested that yeast, and other creatures lacking pairing centers, have two kinds of crossing over, one of which is Msh4 independent, has little or no crossover interference, and serves to

establish effective pairing of homologous chromosomes.

STAHL *et al.* (2004) noted that the concept of two kinds of crossing over provides an explanation for the apparent correlation between the strength of interference and the fraction of crossovers that are Msh4 dependent in a given interval. Furthermore, MALKOVA *et al.* (2004), using a statistical analysis, which in the light of information presented here appears oversimplified, reported that the distribution of crossovers along the left arm of chromosome VII in wild-type yeast was better described by a two-kinds-of-crossover model than by the simple “counting model” for interference (FOSS *et al.* 1993). More compelling support came from the phenotype of *mms4* and *mus81* deletions. Each of these mutations caused a reduction in crossing over but not in interference, while deletion of *MMS4* along with deletion of *MSH4*'s partner, *MSH5*, caused a further reduction in crossing over (DE LOS SANTOS *et al.* 2003). Apparently, the *mms4* and *mus81* mutations specifically reduce Msh4-independent crossing over. However, in otherwise wild-type strains, *mms4/mus81* reductions in crossing over do not appear to reduce chromosome pairing nor do they reduce meiosis I disjunction (DE LOS SANTOS *et al.* 2001, 2003; and see MALOISEL *et al.* 2004). These observations prompt a modification of the influential hypothesis of ZALEVSKY *et al.* (1999): instead of being dependent on Msh4-independent crossovers, chromosome pairing in yeast is dependent on a class of double-strand-break-repair (DSBr) events of which the crossovers happen to

This article is dedicated to the memory of David R. Stadler.

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be relatively Msh4 independent. This framework of thought, similar to that adopted by PEOPLES-HOLST and BURGESS (2005), has guided our analysis.

To test the hypothesis of STAHL *et al.* (2004) that interfering and “non”-interfering crossovers should be distinguishable from each other in wild-type yeast, we measured interference in strains marked (near DSB hotspots at *HIS4* on chromosome III and at *ARG4* on chromosome VIII) with palindromes that make poorly repairable mismatches (PRMs) in heteroduplex DNA, often resulting in 5:3 segregation at the palindrome site. (Throughout, we designate an aberrant segregation as 5:3 or 6:2 without regard to which allele is present in excess.) In the event, our results refute particulars of the hypothesis—identifiable “resolution types” proved indifferent to Msh4—and our concept of ligated *vs.* unligated intermediates of canonical DSB (SUN *et al.* 1991; popularly referred to as DSB intermediates) proved useless. However, our results provide compelling evidence that wild-type yeast has distinct interference and “non”-interference classes of DSB. [The quotation marks on “non”-interference reflect the observations that, in wild type, this class appears to yield crossovers with negative interference (see RESULTS) and that some *msh4* strains show residual positive interference.]

Our observations include evidence that one class of conversions, those with 5:3 segregation at the palindrome site, is characterized by the absence of normal crossover interference. Furthermore, the crossover (and noncrossover) frequencies of 5:3 tetrads are seen to be relatively independent of Msh4 function, implying that there were few, if any, interfering, Msh4-dependent crossovers among tetrads that failed to undergo mismatch repair (MMR) of the marked heteroduplex. This conclusion prompts the deduction that interfering, Msh4-dependent crossovers essentially always undergo such MMR. This concept has provided a framework for dealing with all the observations reported here.

In yeast, most MMR is apparently directed by strand discontinuities. Strand discontinuities are notably present at two stages of DSB: during the process of strand invasion (round one) and during or following any steps required to resolve recombination intermediates (round two; *e.g.*, resolution of Holliday junctions). MMR at invasion (HABER *et al.* 1993) is deemed responsible for the observation that, in yeast, repair of mismatches yielding 6:2 conversions close to the DSB favors markers from the parent that does not suffer the initiating double-strand break but serves as jig and template for the repair of that break. [It was this that misled SZOSTAK *et al.* (1983) to propose gap repair as the major conversion mechanism in yeast.] In yeast, this bias is apparent as well in the short-patch repair that is evident in MMR-compromised conditions (Coïc *et al.* 2000). FOSS *et al.* (1999) presented evidence in support of the idea of a second opportunity for MMR, directed by cuts introduced at Holliday junction resolution.

Our data suggest rules for the repair of PRMs as well as for well repairable mismatches (WRMs) in the two classes of DSB: (1) In the “non”-interference class, PRMs are subject to some repair, but only during the process of invasion; (2) in the interference class, PRMs are invariably repaired, but only as part of the process of the resolution of a joint-molecule intermediate; and (3) in both classes, WRMs close to the DSB are usually repaired at the invasion stage to yield 6:2 segregation of the marker. We will refer to this proposal as “the rules.” We offer the rules not as “eternal truth,” but as a guide for thinking about our results. As far as we know, they contradict no established observations from other investigations, although they seem to lead to views of meiosis that contradict some beliefs. As with all biological rules, nature may sometimes bend them.

For DSB events monitored by a PRM, the rules predict that round two MMR will often erase evidence of the event by restoring normal 4:4 segregation of the diagnostic marker. We tested this prediction by using a marker that makes frequent WRMs close to a DSB hotspot to screen for tetrads with a DSB event. Within that class of tetrads, we tested whether the conversion frequency of a PRM, close by and on the opposite side of the DSB, would be lower than that of a WRM at the same site.

To pursue the attractive proposal of a connection between homolog pairing and the “non”-interference class of DSB, we made use of PRMs to assess whether the DSB phenotypes of the pairing mutant *ndj1* (also known as *tam1*) are preferentially associated with one or the other of the two DSB phases. Deletion of *NDJ1* causes a delay in pairing of homologs (CHUA and ROEDER 1997; CONRAD *et al.* 1997; PEOPLES-HOLST and BURGESS 2005), homolog nondisjunction (CHUA and ROEDER 1997; CONRAD *et al.* 1997), and an apparent reduction in “noncrossovers,” *i.e.*, conversions unaccompanied by crossing over (WU and BURGESS 2006), and in crossover interference without any reduction in crossing over (CHUA and ROEDER 1997). In fact, the published data of CHUA and ROEDER (1997) are compatible with a modest increase in crossing over for the *ndj1* mutants, varying, perhaps, with the interval tested. Here we offer evidence of a specific *ndj1*-induced increase in crossovers that are “non”-interfering as deduced from their segregation pattern. We propose that this increase in crossovers contributes to the *ndj1*-induced decrease in interference reported by CHUA and ROEDER (1997).

We apply the rules to interpret the principal differences among our data, those of MORTIMER and FOGEL (1974) and MALKOVA *et al.* (2004), and those of KITANI (1978), who conducted a similar study in *Sordaria fimicola*, a filamentous fungus that frequently fails to correct mismatches. Together, these studies suggest (1) that “poor repairability” of mismatches used to identify a DSB event allows identification of the “non”-interfering crossovers in wild-type yeast and *Sordaria* and (2) that *Sordaria*, like yeast, relies on a class of DSB characterized

TABLE 1
Yeast strains

Strain	Genotype	Source
AS4	<i>MATα trp1-1 tyr7-1 ade6 ura3-52 arg4-17</i>	STAPLETON and PETES (1991)
AS13	<i>MATa ade6 ura3-52 CAN1 leu2-Bst</i>	STAPLETON and PETES (1991)
F1209	<i>MATα arg4-1691-lop his3-Δ200 lys-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI spo13::URA3-loxP</i>	GILBERTSON and STAHL (1996)
F1210	<i>MATa ARG4::HpaI-lopC his3-Δ200 lys-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI trp1-ΔXbaI DFF::LEU2-loxP</i>	GILBERTSON and STAHL (1996)
F1225	<i>MATa ade2-EcoRV-XhoI leu2-ΔKpnI ura3-52 lys2-HpaI-KpnI trp1-ΔXbaI arg4-1691-lop his3-Δ200</i>	Jasper Rine
F1227	<i>MATα lys2-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI DFF::LEU2-loxP his3-Δ200 spo13::URA3-loxP</i>	Jasper Rine
F1231	<i>MATa/MATα arg4-1691-lop/arg4-BglIII-ClaI his3-Δ200/his3-Δ200 lys2-HpaI-HindIII/lys2-HpaI-HindIII leu2-ΔKpnI/leu2-ΔKpnI ura3-52/ura3-52 ade2-EcoRV-XhoI/ade2-EcoRV-XhoI TRP1/trp1-ΔXbaI DFF::LEU2-loxP spo13::URA3-loxP</i>	Laboratory collection (Rine background)
F1232	<i>MATa arg4-1691-ΔSalI his3-Δ200 lys2-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI trp1-ΔXbaI</i>	Laboratory collection (Rine background)
YFS26	F1210 <i>tam1Δ::KANMX4</i>	This study
YFS27	F1209 <i>tam1Δ::KANMX4</i>	This study
YFS40	Diploid: F1209 × F1210 (<i>NDJ1</i> Rine background)	This study
YFS41	Diploid: YFS26 × YFS27 (<i>ndj1</i> Rine background)	This study
YFS617	F1225 <i>TRP1 ARG4 HIS3 spo13::URA3-loxP YCL033C::NatMX4</i>	This study
YFS618	F1227 <i>his4-IR9 arg4-1691-lop FUS1::KanMX4</i>	This study
YFS621	Diploid: YFS617 × YFS618 (<i>MSH4 NDJ1</i> Rine background)	This study
YFS634	YFS617 <i>msh4Δ::HphMX4</i>	This study
YFS635	YFS618 <i>msh4Δ::HphMX4</i>	This study
YFS636	Diploid: YFS634 × YFS635 (<i>msh4</i> Rine background)	This study
YFS637	<i>MATα arg4 his3-Δ200 lys-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI DFF::LEU2-loxP spo13::URA3-loxP</i>	This study
YFS638	<i>MATα ARG4::HpaI-SalI his3-Δ200 lys-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI DFF::LEU2-loxP spo13::URA3-loxP</i>	This study
YFS639	<i>MATα ARG4::HpaI-lopC his3-Δ200 lys-HpaI-HindIII leu2-ΔKpnI ura3-52 ade2-EcoRV-XhoI DFF::LEU2-loxP spo13::URA3-loxP</i>	This study
YFS641	Diploid: F1232 × YFS638 (WRM, Rine background)	This study
YFS642	Diploid: F1232 × YFS639 (PRM, Rine background)	This study
YFS644	YFS617 <i>tam1Δ::HPHMX4</i>	This study
YFS645	YFS618 <i>tam1Δ::HPHMX4</i>	This study
YFS646	Diploid: YFS644 × YFS645 (<i>ndj1</i> Rine background)	This study
YFS703	AS4 <i>leu2Δ::loxP FUS1Δ::KanMX4 DFF::LEU2 arg4-1691-lop trp1-1Δ::loxP YHR032W::TRP1</i>	This study
YFS706	AS13 <i>his4-IR9 YCL033C::NatMX4 spo13::URA3 TRP1Δ::loxP leu2Δ::loxPΔ::HphMX4</i>	This study
YFS707	Diploid: YFS703 × YFS706 (<i>MSH4</i> Petes background)	This study
YFS711	YFS703 <i>msh4Δ::loxP</i>	This study
YFS712	YFS706 <i>msh4Δ::loxP</i>	This study
YFS713	Diploid: YFS711 × YFS712 (<i>msh4</i> Petes background)	This study

by reduced interference and distinctive MMR to achieve normal homologous pairing.

MATERIALS AND METHODS

Strain construction: Strains bearing markers that make PRMs at the *ARG4* and *HIS4* loci were constructed in two different backgrounds (Table 1). Markers were introduced by standard lithium acetate transformation using either DNA restriction fragments or PCR fragments primed by the oligonucleotides listed in Table 2. Both the PCR and restriction fragments were generated from plasmids described in Table 3.

Previously characterized palindromic markers at *HIS4* (NAG and PETES 1991) and at *ARG4* (GILBERTSON and STAHL 1996)

were introduced by standard two-step transplacement (AUSUBEL *et al.* 1994). The haploid progenitors for the first background were F1225 and F1227, obtained from the laboratory of Jasper Rine (“Rine background”). The haploid progenitors for the second background were strains AS4 and AS13, obtained from the laboratory of Tom Petes (“Petes background”). Deletion of *MSH4* was achieved in the Petes background via the *loxP-Cre* recombinase system and the bleomycin-resistance gene (GÜLDENER *et al.* 1996), leaving a residual *loxP* site. The deletion reduced the frequency of tetrads with four viable spores from 0.76 to 0.67. The hygromycin-resistance gene was used to replace *MSH4* in the Rine background, reducing four-spore-viable tetrads from 0.76 to 0.46.

YFS26 and YFS27 were constructed by transformation (GIETZ *et al.* 1992) of F1209 or F1210 (Rine background) with a 2.2-kb fragment liberated by *NotI* from pYORC-YOL104C. YFS644 and

TABLE 2
Oligonucleotides

Primer	Sequence	Purpose
FS91	5'-CAGAGTTCTGTGCTTCGCTG-3'	Score silent <i>ARG4</i> markers
FS92	5'-GTATCCACGTTTCAGCGGTAG-3'	
FS105	5'-ACG-ACG-AGC-AGT-TAA-AGT-TTT-CAA-ATA-AGT-TGC-AAC-CAG-CAG-ACA-TGA-TAC-GTA-CGC-TGC-AGG-TCG-AC-3'	Replace nucleotides 2190–2340 downstream of the <i>FUS1</i> ORF ATG on chromosome III with <i>KAN-MX4</i>
FS106	5'-TGT-GGC-GTT-TTA-CGT-GAA-AAT-TAC-GTA-AAG-AAA-AAG-ATC-CTG-GGG-TGC-CT-ATC-GAT-GAA-TTC-GAG-CTC-G-3'	
FS110	5'-GTA-ACT-CCG-GTT-TCA-AAG-CG-3'	Confirm structure of <i>KAN</i> insertion
FS111	5'-ACA-ATA-ATC-CAG-TAT-ACC-GC-3'	
FS228	5'-GCA-GCT-GAT-GGT-GCT-GAG-AGA-TAA-GGC-CAC-TGAAAG-GCC-CCG-TAC-GCT-GCA-GGT-CGA-C-3'	Replace nucleotides 190–320 downstream of <i>YCL033C</i> ATG on chromosome III with <i>NatMX4</i>
FS229	5'-ATT-AAA-GAA-TTG-TCA-CGA-TGA-TAT-GTG-ATG-GCT-CCA-GGG-GAT-CGA-TGA-ATT-CGA-GCT-CG-3'	
FS232	5'-TCC-TGG-CAA-TCT-TGC-AAG-CAC-AAT-TCC-GGC-3'	Confirm <i>NAT</i> insertion
FS233	5'-CCA-CGT-CCA-AGT-TCA-TCC-AGG-CAA-GGG-CG-3'	
FS280	5'-ATATT GTCAT GAACT ATACC ATATA CAACT TAGGA TAAAAATACA GGTAG CGTAC GCTGC AGGTC GAC-3'	Delete <i>TAM1</i>
FS281	5'-AACAG CAAAG AAAAG TTTT TTTGG TTCAG ATGTAATATG GATAG CCCGT ATCGA TGAAT TCGAG CTCG -3'	
FS282	5'-GTTTC GTACT CAGTG ACGTA CCGGG-3'	Confirm YFS644 and YFS645
FS283	5'-AAATG CATTC CTACT AACGA ATCGG-3'	
FS284	5'-GAA-GGC-TTT-CCA-ACT-TAA-AAG-AGC-CTC-AAC-3'	Replace <i>MSH4</i> ORF of YFS0617 and YFS0618 with <i>HphMX4</i> ORF
FS285	5'-GTT-TTG-GTA-TGG-GAT-GAC-ATT-GTT-TTA-CGT-AG-3'	
FS288	5'-ATC-AAG-CAG-CAG-TAC-CGG-TAT-CTC-AAG-AGG-3'	Confirm structure of <i>HYG</i> insertion
FS294	5'-ACA-TTT-CAG-CAA-TAT-ATA-TAT-ATA-TAT-TTC-AAG-GAT-ATA-CCA-TTC-CGT-ACG-CTG-CAG-GTC-GAC-3'	Amplify hygromycin-resistance gene for insertion at the native <i>LEU2</i> site
FS295	5'-TTC-ATT-TAT-AAA-GTT-TAT-GTA-CAA-ATA-TCA-TAA-AAA-AAG-AGA-ATC-ATC-GAT-GAA-TTC-GAG-CTC-G-3'	
FS300	5'-GAG-CTA-GGT-GGT-GTT-ACA-CTC-GGT-TCT-ATG-ACT-GCT-AAC-ATC-ACG-GCG-ACA-TTA-CTA-TAT-ATA-TAA-TAT-AGG-3'	Introduce <i>TRP1</i> upstream of <i>ARG4</i> in vicinity of <i>YHR032W</i>
FS301	5'-ACC-AAA-CAT-ACA-TCA-TTG-GCA-AGA-ACG-CCA-AGA-TGG-TGA-TCA-CAG-CCA-AAC-AAT-ACT-TAA-ATA-AAT-ACT-ACT-CAG-3'	
FS302	5'-GTG-AGT-ATA-CGT-GAT-TAA-GCA-CAC-AAA-GGC-AGC-TTG-GAG-TTC-GAC-AAC-CCT-TAA-TAT-AAC-TTC-G-3'	Amplify bleomycin-resistance gene
FS303	5'-TGC-ACA-AAC-AAT-ACT-TAA-ATA-AAT-ACT-ACT-CAG-TAA-TAA-CTC-GAC-AAC-CCT-TAA-TAT-AAC-TTC-G-3'	

YFS645 were made by deletion of *TAM1* in YFS617 and YFS618 was made by replacement with the *HPHMX4* ORF of pAG32 using primers FS280 and FS281. Confirmation of the insertion was made by PCR, using primers FS282 and FS283. YFS637 is a meiotic segregant of F1231. YFS638 and YFS639 were generated by transforming YFS637 with *HindIII*–*EcoRI* fragments of pLG56 (*ARG4::HpaI-SalI*) and pLG57 (*ARG4::HpaI-lopC*), respectively. For each strain, Arg⁺ transformants were screened by PCR with primers FS91 and FS92, and the presence of the correct *ARG4* allele was verified by restriction analysis. The 398-nucleotide (nt) PCR fragment containing the silent *ARG4::HpaI-SalI* allele is labile to *SalI* digestion and resistant to *HpaI* and *SpeI* digestion. The 424-nt PCR fragment containing the silent *ARG4::HpaI-lopC* allele contains a *SpeI* site within the *lopC* palindrome.

The reported locations of the *ARG4* and *HIS4* double-strand-break sites (NICOLAS *et al.* 1989; FAN *et al.* 1995) were assumed to apply to our strains.

Genetic analyses: *Data:* Data were tabulated and analyzed with the aid of the MacTetrad 6.9.1 program available from Gopher at merlot.wekj.jhu.edu. The high rates of conversion at *HIS4* and *ARG4*, indicative of high rates of DSBs, inevitably led to multiple-event four-spore viable tetrads that could not be included in some analyses. Standard statistical analyses were conducted with the aid of the online calculators at VassarStats. Tetrad-specific statistical analyses were carried out with calculators at Stahl Lab Online Tools (<http://molbio.uoregon.edu/~fstahl/>). All *P*-values are reported without regard to the number of analyses performed.

TABLE 3

Plasmids

Gene	Plasmid	Purpose	Source
<i>HIS3</i>	pJJ217	<i>HIS3</i> to replace <i>his3-Δ200</i> in F1225 and F1227	JONES and PRAKASH (1990)
<i>NatMX4</i>	pAG25	Nourseothricin-resistance gene inserted into YCL033C near <i>HIS4</i> in YFS617	GOLDSTEIN and McCUSKER (1999)
<i>KanMX4</i>	pFA6 <i>KanMX4</i>	Source of kanamycin-resistance gene in YFS618 and YFS703	GÜLDENER <i>et al.</i> (1996)
<i>his4-IR9</i>	pDN22	Poorly repairable marker introduced into YFS618 and YFS706	NAG and PETES (1991)
<i>HphMX4</i>	pAG32	Hygromycin-resistance gene replaces <i>MSH4</i> in YFS617 and YFS618; inserted at <i>LEU2</i> in YFS706 and YFS712	GOLDSTEIN and McCUSKER (1999)
<i>BleMX4</i>	pUG66	Bleomycin-resistance gene replaces <i>MSH4</i> by <i>loxP</i> in YFS703 and YFS706	GÜLDENER <i>et al.</i> (1996)
<i>URA3</i>	pLG54	Source of <i>URA3</i> gene inserted into <i>SPO13</i> near the <i>ARG4</i> locus in YFS617 and YFS706	GILBERTSON and STAHL (1996)
<i>arg4-1691-lop</i>	pLG55	Poorly repairable marker introduced into YFS618 and YFS703	GILBERTSON and STAHL (1996)
<i>TRP1</i>	pRS304	Source of <i>TRP1</i> gene introduced at YHR032W in YFS703 and YFS711	SIKORSKI and HIETER (1989)
<i>tam1Δ::KANMX4</i>	pYORC-YOL104C	Delete <i>TAM1</i> in F1209 and F1210	GOLDSTEIN and McCUSKER (1999)
<i>HPHMX4</i>	pAG32	Delete <i>TAM1</i> in YFS617 and YFS618	
<i>ARG4::HpaI-SalI</i>	pLG56	Insert <i>ARG4::HpaI-SalI</i> into YFS637	GILBERTSON and STAHL (1996)
<i>ARG4::HpaI-lopC</i>	pLG57	Insert <i>ARG4::HpaI-lopC</i> into YFS637	GILBERTSON and STAHL (1996)

Map lengths: Map length (in centimorgans) for any interval, defined as 100 times the mean number of exchanges per meiosis, was calculated according to PERKINS (1949). Map length can be calculated only when neither marker defining the interval undergoes conversion.

Interference: In some analyses, the map length of an interval (PERKINS 1949) was compared for populations that did, or did not, have a crossover in an adjacent interval. A significant difference in map length (two-tailed $P < 0.05$) due to the crossover in the adjacent interval conservatively indicates

TABLE 4

MAT-KAN* and *HYG-KAN* map distances among crossovers and noncrossovers in the adjacent *KAN-HIS4-NAT* interval in tetrads with 6:2, 5:3, or normal 4:4 segregation for *HIS4

Event in <i>KAN-HIS4-NAT</i> interval	<i>MAT-KAN</i> ^a distance (cM); PD/NPD/TT	<i>HYG-KAN</i> ^b distance (cM); PD/NPD/TT
Crossovers plus noncrossovers	36.7 ± 1.1; 1042/76/1377	5.1 ± 0.4; 2185/4/223
Crossovers	30.8 ± 2.4; 225/11/206	2.8 ± 0.6; 760/2/33
Noncrossovers	38.0 ± 1.2; 817/65/1171	6.3 ± 0.5; 1425/2/190
4:4 crossovers	29.3 ± 2.3 ^{c,d} ; 193/7/181	1.9 ± 0.4 ^{c,d} ; 530/0/21
4:4 noncrossovers	37.6 ± 1.2; 792/60/1130	6.3 ± 0.5; 1256/1/175
Conversion crossovers	40.2 ± 9.3; 32/4/25	4.9 ± 1.9; 230/2/12
Conversion noncrossovers	50.0 ± 8.6; 25/5/41	5.7 ± 1.9; 169/1/15
6:2 crossovers	20.8 ± 5.0 ^{c,e} ; 14/0/10	1.4 ± 0.9 ^c ; 72/0/2
6:2 noncrossovers	66.7 ± 20.0; 4/2/12	10.9 ± 9.3; 30/1/1
5:3 crossovers	52.7 ± 14.7; 18/4/15	6.5 ± 2.6; 158/2/10
5:3 noncrossovers	44.3 ± 9.2; 21/3/29	4.6 ± 1.2; 139/0/14

Significance ($P \leq 0.05$) was determined as described in MATERIALS AND METHODS.

^a Rine background: normal 4:4 for *MAT*, *KAN*, *NAT*.

^b Petes background: normal 4:4 for *HYG*, *KAN*, *NAT*.

^c Significantly different from total classifiable tetrads and from total noncrossovers.

^d Significantly different from 4:4 noncrossovers.

^e Significantly different from 6:2 noncrossovers.

^f Significantly different from 6:2 crossovers.

interference. Tests for significance of difference between two Perkins map lengths were conducted with the aid of Stahl Lab Online Tools. In Table 4, all such tests that indicated a significant difference in map lengths were confirmed by a Monte Carlo simulation as follows.

To determine whether two genetic distances were statistically distinguishable or not using a permutation test, we first pooled the parental ditypes (PDs), tetratypes (TTs), and nonparental ditypes from the two intervals. Then, for 1000 simulations, we randomly distributed the pooled types back into two randomized data sets, keeping the original total number of tetrads in each data set. The approximate *P*-value for this permutation test is the proportion of the 1000 randomized data sets with a standardized absolute difference in length, $|X_1 - X_2| / (SE(X_1 - X_2))$, that was at least as large as that observed in the original data.

In other analyses, interference was detected as a shortage of multiple exchanges as indicated by a nonparental ditype (NPD) ratio significantly less than unity (PAPAZIAN 1952). When wild-type interference is compared with interference in a mutant that has very different map lengths, *m*, an index of interference that is independent of map length (STAHL and LANDE 1995), was determined using the *m* calculator at Stahl Lab Online Tools. Beginning with FOSS *et al.* (1993) and MCPHEE and SPEED (1995), this model has proven to be a useful description of interference.

Msh4 crosses: For crosses in the Rine background, diploids YFS621 and YFS636 (Figure 1) were streaked onto YEPD, grown for 2 days at 30°, patched onto YEPD, and incubated for 1 day at 30°. The patches were then replica printed to sporulation medium (MALKOVA *et al.* 2004) and incubated for 3 days at 30°. Asci were dissected onto 2× YEPD and incubated for 5 days at 30°. For crosses in the Petes background, diploids YFS707 and YFS713 (Figure 1) were streaked onto rich medium, grown for 2 days at 30° and then inoculated into 50 ml YEPD in a 500-ml flask and aerated at 300 rpm for 1 day at 30°. Cells were then diluted to an A_{600} of 2.5, washed once with water, and resuspended in a 250-ml flask in 25 ml sporulation medium with amino acids at 1/5 the standard concentrations for growth (HILLERS and STAHL 1999). They were then incubated for 5 days at 18° with aeration at 300 rpm. Asci were collected, washed, and dissected on 2× YEPD plates (HILLERS and STAHL 1999). After incubation for 5 days at 30°, the dissection plates were replica printed to determine segregation patterns. Our ability to score conversions correctly was confirmed by picking and replating an appropriate number of colonies that had been identified as 5:3 or 6:2 conversions.

PRM vs. WRM study: Sporulation of YFS641 and YFS642 (Figure 2) was performed as in GILBERTSON and STAHL (1996) except that diploid cells were grown for only 1 day on YEPD prior to replica printing onto sporulation medium. Tetrads were dissected onto 2× YEPD and then incubated at 30° for 5 days. The tetrads were then printed to plates containing standard arginine, leucine, and uracil omission media.

For screening tetrads by PCR, the tetrads were printed to fresh YEPD and incubated overnight (only) at 30°. Each entire colony was lifted with a plastic pipette tip and suspended directly into a 30-μl PCR reaction mix containing 300 μM dNTPs, 2.5 units TAQ polymerase (Promega, Madison, WI), 2 mM MgCl₂, primers FS91 and FS92 at 640 pmol, all in Promega 1× reaction buffer. FS91 and FS92 amplify a fragment from –506 nt through –108 nt, upstream of the start of the *ARG4* ORF. The resulting PCR reactions were screened for the respective silent *ARG4* alleles by restriction digestion (as above) and electrophoresis. The *ARG4::HpaI-lopC* allele yields a 424-bp fragment readily distinguishable from both the wild-type *ARG4* and *ARG4::HpaI-SalI* alleles (each 398 bp in length) by electrophoresis on 3% NuSieve GTG low-melting-point aga-

rose gels run in 1× TBE buffer at room temperature. To ensure proper scoring of the *ARG4::HpaI-SalI* allele, PCR reactions were split in two. One aliquot was digested with *SalI* and the other with *HpaI* prior to gel electrophoresis. The reliability of detection of 5:3 conversions was confirmed by the procedure described in HOFFMANN *et al.* (2005); all 41 reconstructed colonies tested positive for sectoring of the PRM.

The intended properties of the three markers closely bracketing the DSB site were confirmed by randomly testing tetrads from each of the two crosses. Of 100 tetrads, the silent marker making WRMs (*HpaI-SalI*) had nine 6:2 conversions and no 5:3 conversions, as did the *arg4* marker. All conversions were co-conversions. Of 105 tetrads, the silent marker making PRMs (*lopC*) had five 6:2 conversions and seven 5:3 conversions. Of the five 6:2 conversions, three were accompanied by 6:2 conversion at *ARG4*. Of the seven 5:3 conversions, four were accompanied by 6:2 conversion at *ARG4* (in one of these tetrads, the two conversions were in favor of different parents), and three were 4:4 at *ARG4*. In all cases, except the one noted, the two associated conversions were in favor of the same parent (*i.e.*, co-conversions). In this set of 105 tetrads, the *arg4* marker enjoyed nine 6:2 conversions and no 5:3 conversions.

Ndj1 study: Diploid strains (Rine background) YFS40 and YFS41 (Figure 3) were streaked from the –70° freezer onto YEPD and grown for 3 days at 30°. Single colonies were then patched onto YEPD and incubated for 1–2 days at 30°. The patches were replica printed to YEPEG (AUSUBEL *et al.* 1994) for 2–3 days at 30° and then replica printed to KOAC (MCCUSKER and HABER 1988) for 5 days at 25°. Tetrads were dissected to YEPD and grown for 5 days at 30°.

Diploid strains (Rine background) YFS621 (Figure 1) and YFS646 were streaked onto YEPD and grown for 2 days at 30°. YFS621 data are from the Msh4 study. Single colonies were then patched onto YEPD plates and incubated for 1 day at 30°. The patches were then replica printed to sporulation medium containing ampicillin (100 mg/liter) for 3 days at 30°. Tetrads were dissected on YEPD plates and grown for 5 days at 30°. The tetrad colonies were replica printed to the appropriate omission or antibiotic media to determine the phenotypes. The *NDJ1* strains YFS40 and YFS621 yielded different ratios of 6:2/5:3 tetrads at *ARG4* (*P* = 0.01). The conclusions that we draw from our analyses are insensitive to that ratio.

RESULTS

Experimental approach: Our crosses were designed to yield a high rate of conversion (to facilitate data collection) with a large proportion of 5:3's (to detect heteroduplexes). For most of these crosses, full data sets have been deposited as supplemental material. For each of two loci (*ARG4* and *HIS4*) in each of two strain backgrounds ("Rine" and "Petes": Figure 1), we analyzed meiotic tetrads in which each DSB hotspot was marked with a small palindrome that makes PRMs (NAG *et al.* 1989) in heteroduplex DNA. The high rate of conversion resulted in numerous tetrads that had evidently enjoyed multiple events. These tetrads were necessarily excluded from some analyses. A DSB event is recognizable as a gene conversion if either repair of the resulting mismatch failed (resulting in 5:3 segregation of the marker) or the mismatch was repaired in favor of the duplex that was invaded, as defined by the DSB model (yielding 6:2 segregation). Repair of the mismatch in favor of the in-

FIGURE 1.—Diagrams of Rine background YFS621 (*MSH4*) and YFS636 (*msh4*) and Petes background YFS707 (*MSH4*) and YFS713 (*msh4*) diploids employed in the Msh4 studies (Tables 4–8). The data from the diploid YFS621 were also used in the NDJ1 studies (Tables 11–13). Distances are in kilobases.

vading strand will restore normal 4:4 segregation of the marker, thereby erasing the incipient gene conversion. To allow us to detect crossing over, we bracketed each hotspot with two closely linked, conveniently scored insertions. In addition, the mating-type locus (*MAT*) in the Rine background and the inserted drug-resistance marker *HYG* in the Petes background defined intervals adjacent to the *HIS4*-containing interval to be used for assessing interference (Figure 1).

5:3 crossovers lack positive interference: To assess interference between DSB_r events in the *KAN-HIS4-NAT* interval and crossovers in the adjacent *MAT-KAN* or *HYG-KAN* interval, we measured, for each segregation class as defined with respect to *HIS4* conversion, the effect of a crossover or a noncrossover in the *KAN-HIS4-NAT* interval on the map length (centimorgans) of the *MAT-KAN* or *HYG-KAN* interval. A reduction or increase in the latter relative to those in the remaining population of classifiable tetrads indicates positive or negative interference, respectively. Table 4 shows that conversion

crossovers, as a class, failed to manifest interference. [This behavior differs from that reported for WRMs by MORTIMER and FOGEL (1974) and MALKOVA *et al.* (2004); see *Testing the rules* in the DISCUSSION.] However, the 5:3 crossovers as a class are seen to differ from the 6:2 and 4:4 crossovers. Specifically, the 5:3 crossovers appear to manifest negative interference, whereas the 6:2 and 4:4 crossovers display positive interference.

We take these results to be a demonstration that wild-type yeast does have both interference and “non”-interference crossovers, which, by means of their different MMR properties, can be demonstrated without the involvement of recombination-disrupting mutations. According to the “two-pathway model” of ZALEVSKY *et al.* (1999), the 5:3 crossovers must have arisen from the Msh4-independent, “non”-interference class of DSB_r. That view predicts that deletion of *MSH4*, which does reduce both crossing over (Table 5) and interference (Table 6) in most intervals of our strains, should reduce the frequency of 6:2 and 4:4 crossovers but not the

TABLE 5
Map distances in *MSH4* and *msh4* strains

	<i>MAT-KAN</i>	<i>KAN-NAT</i>			<i>LEU-URA^a</i>
A. Rine background					
<i>MSH4</i> (YFS621)	36.9 ± 1.0		8.9 ± 0.4		12.6 ± 0.5
<i>msh4</i> (YFS636)	17.2 ± 0.9		5.0 ± 0.4		6.3 ± 0.6
	<i>MAT-KAN</i>	<i>HYG-KAN</i>	<i>KAN-NAT</i>	<i>TRP-LEU^a</i>	<i>LEU-URA^a</i>
B. Petes background					
<i>MSH4</i> (YFS707)	41.3 ± 1.2	4.9 ± 0.3	19.2 ± 0.7	4.3 ± 0.3	13.3 ± 0.5
<i>msh4</i> (YFS713)	19.4 ± 1.0	2.2 ± 0.3	9.8 ± 0.7	2.0 ± 0.3	6.9 ± 0.5

Map distances (in centimorgans) are from PERKINS's (1949) equation, which underestimates long distances.

^a These intervals, like those with drug resistance markers, are defined by inserts. The *TRP* insertion, included here for its contribution to these linkage data, proved to be too close, in centimorgans, to the *LEU-ARG4-URA* interval to give useful interference data for inclusion in Table 4.

TABLE 6
Interference as *m*; NPD ratio; and observed PD/NPD/TT in *MSH4* and *msh4* strains

	<i>MSH4</i>	<i>msh4</i>
<i>LEU-URA</i> (Rine)	1, 2; 0.16 ± 0.08; 2128/4/683	0; 1.28 ± 0.74; 1225/3/155
<i>LEU-URA</i> (Petes)	1; 0.33 ± 0.11; 2218/9/734	0; 0.26 ± 0.26; 1344/1/207
<i>KAN-NAT</i> (Rine)	0, 1; 0.18 ± 0.13; 2108/2/444	NA; 0/0.001; 1082/0/118
<i>KAN-NAT</i> (Petes)	1; 0.37 ± 0.09; 1642/18/861	0; 0.88 ± 0.40; 1086/5/230
<i>TRP-URA</i> (Petes)	1; 0.30 ± 0.08; 2011/15/951	0; 0.76 ± 0.34; 1301/5/270
<i>MAT-KAN</i> (Rine)	1; 0.46 ± 0.05; 1120/84/1475	0, 1; 0.40 ± 0.14; 898/8/403
<i>MAT-KAN</i> (Petes)	0.5%; 0.72 ± 0.07; 1141/131/1505	0; 0.70 ± 0.17; 981/17/467
<i>MAT-NAT</i> (Rine)	1; 0.49 ± 0.05; 898/121/1564	1; 0.43 ± 0.12; 736/14/479
<i>MAT-NAT</i> (Petes)	1; 0.64 ± 0.06; 787/189/1658	0; 0.73 ± 0.13; 762/33/583

m-Values (see MATERIALS AND METHODS) were determined at Stahl Lab Online Tools. The values entered are those with which the data are compatible (95% confidence). Any entry that does not include *m* = 0 is indicative of statistically significant interference.

"The 95% confidence envelope for this entry intersects none of the *m* curves, but falls about halfway between the curves for *m* = 0 and *m* = 1.

frequency of crossovers with 5:3 segregation for the palindrome site.

Deletion of *MSH4* reduces primarily 4:4 and 6:2 crossovers: We tested the above prediction (Table 7) with a set of diploids that are isogenic to those described, except for deletion of the *MSH4* gene. Deletion of *MSH4* had a minor effect on crossovers with 5:3 segregation at the palindrome site at *HIS4* or *ARG4* in the Petes background, but strongly reduced crossovers with 6:2 or 4:4 segregation.

In the Rine background, the *msh4* mutants displayed an overall increase in conversion rates of the non-palindrome markers (23.5/16.0 = 1.5-fold, Table 8), reflected in an increase (1.6-fold) in the ("Msh4 independent") 5:3 conversions at *ARG4* and *HIS4* in Table 8. *msh4*-induced increases in conversion have been seen previously (ROSS-MACDONALD and ROEDER 1994) but have been downplayed (ROEDER 1997; NOVAK *et al.* 2001). Despite the increased conversion in the Rine strain, 6:2 crossover conversions were reduced significantly at *ARG4* and were *not increased* at *HIS4*, in contrast with the 5:3 conversion crossovers (Table 7). Thus, the Rine data show the same kind of differential effect on the 6:2 *vs.* 5:3 crossovers as do the Petes data.

The effect of *msh4* on the frequencies of crossover tetrads with 5:3, 6:2, or 4:4 segregation of the palindrome sites is quantified for the Petes strains as the percentage of change (Table 7). For the two loci, the *msh4*-induced changes in frequency of 5:3 crossovers average −8%. In contrast, the average value for the 6:2 crossovers is −50% (*P* = 0.02) and for 4:4 crossovers is −55% (*P* < 0.0001).

These data argue that one class of crossovers, which often fails to repair a PRM, occurs with relatively little dependence on Msh4 and displays no positive interference in a *MSH4* background; the other, which rarely, if ever, fails to enjoy such repair, is strongly Msh4 dependent and displays positive crossover interference.

***msh4*-induced increase in noncrossovers:** Table 8 shows that, in the Petes background, the combined frequencies of conversion for the markers with WRMs (all markers except those at *HIS4* and *ARG4*) were unaffected by the *msh4* mutation (26.2% *vs.* 26.3%), as expected. The expectation failed, however, for each of the markers making PRMs. At *HIS4*, 21.3% for *MSH4* fell to 18.3% for *msh4* (*P* = 0.02). At *ARG4*, the corresponding values are 8.9% *vs.* 7.0% (*P* = 0.02). The *msh4*-induced reductions in conversion for *HIS4* and *ARG4* (21.3 − 18.3 = 3.0 and 8.9 − 7.0 = 1.9 percentage points, respectively) are comparable to the *msh4*-induced reductions in 6:2 crossovers (Table 7) for *HIS4* and *ARG4* (1.8 and 1.9 percentage points, respectively). The lost 6:2 crossovers appear to be accommodated by increases in 4:4 noncrossovers, which were greater than the reductions in 4:4 crossovers; for *HIS4* this net increase is 1.6 points, and for *ARG4* the net increase is 1.8 points. Thus, these data (Tables 7 and 8) support our expectation of no net change in conversion frequency for markers making WRMs, but imply that the potential crossovers with 6:2 segregation for markers with PRMs were lost, not only as crossovers but also as conversions, as a result of the *msh4* deletion.

The observation that the markers making WRMs suffered no *msh4*-induced reduction in conversion rates argues against *msh4*-induced, sister-chromatid-dependent DSBs as the cause of reduced conversion associated with PRMs.

The data for *HIS4* in the Petes strain (Table 7) suggest that the modest loss of 5:3 crossovers is compensated by an increase in 5:3 noncrossovers.

Evidence for MMR-dependent restoration of 4:4 segregation for palindromic markers: As described above, tetrads with 4:4 segregation at *HIS4* or *ARG4* enjoyed a net increase associated with deletion of *MSH4* (Tables 7 and 8, Petes background), and this increase was in the noncrossover class. This invites the proposal that, in wild-type yeast as well, 4:4 noncrossovers are

TABLE 7
msh4-induced changes in percentage (and number) of crossovers and noncrossovers for intervals containing *ARG4* or *HIS4* among tetrads with 5:3, 6:2, or normal 4:4 segregation for the relevant palindrome site

	5:3			6:2			Normal 4:4		
	CO	NCO	Σ	CO	NCO	Σ	CO	NCO	Σ
A. Petes: <i>ARG4</i>									
<i>MSH4</i> YFS707	0.64 (19)	0.44 (13)	1.08 (32)	4.73 (140)	2.64 (78)	7.37 (218)	19.65 (581)	71.90 (2126)	91.55 (2707)
<i>msh4</i> YFS713	0.65 (10)	0.32 (5)	0.97 (15)	2.84 (44)	2.84 (44)	5.68 (88)	9.94 (154)	83.42 (1293)	93.35 (1447)
<i>P^a</i>	1	0.6	0.7	0.003	0.7	0.03	<0.0002	<0.0002	0.03
Change	+0.01			-1.89			-9.71	11.52	+1.81
% change	+1.6			-40.0			-49.4		
B. Petes: <i>HIS4</i>									
<i>MSH4</i> YFS707	7.11 (173)	6.29 (153)	13.40 (326)	3.04 (74)	1.36 (33)	4.40 (107)	22.90 (557)	59.29 (1442)	82.20 (1999)
<i>msh4</i> YFS713	5.83 (75)	8.01 (103)	13.84 (178)	1.24 (16)	1.09 (14)	2.33 (30)	9.18 (118)	74.65 (960)	83.83 (1078)
<i>P^a</i>	0.1	0.05	0.7	0.0007	0.5	0.002	<0.0002	<0.0002	0.21
Change	-1.28			-1.80			-13.72	+15.36	+1.64
% change	-18.0			-59.2			-59.9		
C. Rine: <i>ARG4</i>									
<i>MSH4</i> YFS621	2.56 (72)	4.80 (135)	7.35 (207)	3.30 (93)	2.13 (60)	5.44 (153)	18.54 (522)	68.67 (1933)	87.21 (2455)
<i>msh4</i> YFS636	3.26 (45)	7.24 (100)	10.49 (145)	1.16 (16)	5.50 (76)	6.66 (92)	7.02 (97)	75.83 (1048)	82.85 (1145)
<i>P^a</i>				<0.0002			<0.0002	<0.0002	
D. Rine: <i>HIS4</i>									
<i>MSH4</i> YFS621	1.49 (38)	2.15 (55)	3.64 (93)	0.94 (24)	0.70 (18)	1.64 (42)	15.04 (384)	79.68 (2035)	94.71 (2419)
<i>msh4</i> YFS636	2.50 (30)	5.25 (63)	7.74 (93)	0.58 (7)	0.50 (6)	1.08 (13)	6.83 (82)	84.35 (1013)	91.17 (1095)
<i>P^a</i>				0.3			<0.0002	0.0006	

Classifiable tetrads, drawn from the following numbers of four-spore-viable tetrads: Petes *MSH4*, 3037; Petes *msh4*, 1596; Rine *MSH4*, 2876; Rine *msh4*, 1424. Percentages are of classifiable tetrads. Crossovers are TT + NPD. Noncrossovers are PD. CO, crossovers; NCO, noncrossovers.

^aTwo-tail probability that the difference between the two independent proportions could be due to chance alone.

TABLE 8
Conversion frequencies in *MSH4* and *msh4* backgrounds for markers making WRMs or PRMs

	<i>MAT</i>	<i>HYG</i>	<i>KAN</i>	<i>HIS</i>	<i>NAT</i>	<i>TRP</i> ^a	<i>LEU</i>	<i>ARG</i>	<i>URA</i>	WRM ^b
A. YFS707: Petes, <i>MSH4</i>										
Normal 4:4	2979	2975	2829	2389	2682	3003	2985	2768	3009	
8:0	0	0	4	0	11	0	4	3	1	20
6:2	58	62	204	156	344	34	48	230	27	777
5:3	0	0	0	452	0	0	0	35	0	0
Aberrant 4:4	0	0	0	40	0	0	0	1	0	
Σ ^c	58	62	208	648	355	34	52	269	28	797
% conversion	1.9	2.0	6.8	21.3	11.7	1.1	1.7	8.9	0.9	26.2
N = 3037 four-spore viable tetrads; total dissected = 4000										
B. YFS713: Petes, <i>msh4</i>										
Normal 4:4	1568	1566	1488	1304	1402	1582	1558	1485	1588	
8:0	0	0	1	0	4	0	0	2	0	5
6:2	28	30	107	45	190	14	38	93	8	415
5:3	0	0	0	226	0	0	0	16	0	0
Aberrant 4:4	0	0	0	21	0	0	0	0	0	0
Σ ^c	28	30	108	292	194	14	38	111	8	420
% conversion	1.8	1.9	6.8	18.3	12.2	0.9	2.4	7.0	0.5	26.3
P ^d	0.7	0.7	0.9	0.02	0.6	0.4	0.1	0.02	0.1	
N = 1596 four-spore viable tetrads; total dissected = 2400										
C. YFS621; Rine, <i>MSH4</i>										
Normal 4:4	2803		2744	2705	2647		2825	2499	2861	
8:0	0		1	0	3		0	0	0	4
6:2	31		131	60	226		51	164	15	454
5:3	0		0	111	0		0	213	0	0
Aberrant 4:4	0		0	0	0		0	3	0	
Σ ^c	31		132	171	229		51	377	15	459 ^e
% conversion	1.1		4.6	6.1	8.0		1.8	13.2	0.6	16.0
N = 2876 (2834 for <i>MAT</i>) four-spore viable tetrads; total dissected = 4020										
D. YFS636; Rine, <i>msh4</i>										
Normal 4:4	1397		1335	1281	1251		1387	1169	1416	
8:0	0		3	0	7		1	1	0	11
6:2	27		86	30	166		36	101	8	323
5:3	0		0	113	0		0	153	0	0
Aberrant 4:4	0		0	3	0		0	3	0	
Σ ^c	27		89	143	173		37	255	8	334
% conversion	1.9		6.3	10.3	12.1		2.6	18.1	0.6	23.5
N = 1576 four-spore viable tetrads; total dissected = 3000										

^aThe *TRP* insertion, included here for its contribution to these conversion data, proved to be too close, in centimorgans, to the *LEU-ARG4-URA* interval to give useful interference data.

^bSum for the row minus conversions for the markers (at *HIS* and *ARG*) making PRMs.

^cSum of conversions, including aberrant 4:4's.

^dThe probability that the differences in conversion frequencies between the *MSH4* and *msh4* crosses are attributable to chance alone.

^eRaised by one to normalize for tetrads lost in the *MAT* column.

created by round two MMR. This proposal is in harmony with the rules (see Introduction) stating that products of the interference class are subject to MMR-dependent restoration of 4:4 segregation.

To test whether repair of PRMs close to a DSB hotspot in fact does result in frequent 4:4 segregation for the palindrome site, we conducted two crosses in which the DSB hotspot at *ARG4* was marked with an *arg4* mutation that makes WRMs, resulting in a high frequency of 6:2

conversions. This *arg4* marker, located a nominal 190 bp to the "right" of the DSB site (*arg4::1691-ΔSalI*, Figure 2), should signal all or most of the DSB events at *ARG4* that involved homologs. [We consider it likely that most of the conversions are a consequence of DSBs at the *ARG4* hotspot. DSB events originating from the *DED81* break site, >2 kb distant to the left, would generally have resulted in normal 4:4 segregation rather than 6:2 segregation of the marker (Foss *et al.* 1999).] Markers on the

FIGURE 2.—Diagram of the Rine background diploids employed in the PRM *vs.* WRM study (Tables 9 and 10). Δ SalI is an *arg4* marker that makes WRMs to the right of the DSB site. *HpaI*, at the left of the DSB site, is a native restriction site. In the diploid with the phenotypically silent marker that makes a WRM at the left of the DSB site (YFS641), the native *HpaI* restriction site was changed to a *SalI* site. In the diploid with the silent marker that makes a PRM at the left of the DSB site (YFS642), a *lopC* palindrome was inserted into that new *SalI* site. Location of the DSB site is nominal on the basis of NICOLAS *et al.* (1989).

left side of the *ARG4* DSB site were designed to detect the influence of MMR. One of the crosses had a marker (*ARG4::HpaI-lopC*, Figure 2) that makes PRMs at a “silent” (nonauxotrophic) site a nominal 130 bp to the left of the DSB site, while the other had a marker (*ARG4::HpaI-SalI*, Figure 2) making WRMs at the silent site. The silent markers were scored by restriction analysis of PCR-amplified DNA, as described in MATERIALS AND METHODS. It is an important feature of these constructs that the WRMs are close enough to the DSB site that they will usually be subject to invasion-directed repair and, consequently, unavailable for resolution-directed repair, which can result in restoration of normal 4:4 segregation (FOSS *et al.* 1999; HILLERS and STAHL 1999; STAHL and HILLERS 2000). In both crosses, *LEU2* and *URA3* insertions bracketing the DSB site allowed us to detect crossing over associated with conversion at *ARG4*. To screen for conversion at *ARG4* (6:2 in favor of either *ARG4* or *arg4*; see MATERIALS AND METHODS), we replicated the colonies from dissected tetrads to arginine-drop-out plates. The tetrads that exhibited a conversion event were then scored for the silent marker.

The data in Table 9 show that, in the cross with the PRMs at the silent site, tetrads with a conversion on the right side of the DSB often (25/49) lacked conversion on the left side of the DSB. In contrast, in the cross with WRMs on both sides of the DSB, tetrads with a conversion on the right side of the DSB usually (35/40) manifested conversion on the left side as well (see also HOFFMANN *et al.* 2005). This degree of “two-sidedness” is higher than that noted in the pioneering article by SCHULTES and SZOSTAK (1990), probably because their markers were farther from the DSB than the ones used here. Our data demonstrate that a major fraction of DSB events indicated by conversion of a marker that makes WRMs fails to result in conversion for a marker that makes PRMs at the same site. At the same time, the

TABLE 9

Frequency of 4:4 segregation of the silent marker among tetrads with conversion for *arg4*

Silent marker	4:4 segregation
PRM	25/49
WRM	5/40
Significance ^a	$P < 0.0001$

^a One-tailed z-test.

~12% of one-sided events observed in the WRM cross suggests some structurally lopsided DSB events (*e.g.*, ALLERS and LICHTEN 2001).

In >90% of the tetrads identified as conversions for the *arg4* marker to the right of the DSB (Figure 2), both of the bracketing markers, *LEU* and *URA*, segregated 4:4, allowing each of these tetrads to be scored as either a crossover or a noncrossover. Table 10 shows that both crossovers and noncrossovers have a high rate of conversion (15/17 and 19/22, respectively) for the marker making WRMs at the silent site. In contrast, only 11/17 crossovers and 11/29 noncrossovers were converted for the silent marker making PRMs. The greater failure of conversion for noncrossovers than for crossovers was significant and in harmony with results reported by GILBERTSON and STAHL (1996), MERKER *et al.* (2003), and JESSOP *et al.* (2005).

Phenotypes of the *ndj1* mutant: The identification of a “non”-interference class of DSB, proposed to facilitate homologous pairing, prompted us to examine the phenotypes of the *ndj1* mutant. This mutant, named after its meiosis I nondisjunction phenotype (CHUA and ROEDER 1997; CONRAD *et al.* 1997; but see DISCUSSION and supplemental Figure S1), delays homolog pairing

TABLE 10

Segregation of silent markers in crossover and noncrossover conversions of *arg4*

Silent marker	Crossovers		Noncrossovers	
	Normal 4:4	Conversion	Normal 4:4	Conversion
PRM	6	11 ^a	18	11 ^{b,c}
WRM	2	15 ^d	3	19 ^d

The map distances for the bracketing interval *LEU-URA* were 11.8 ± 1.1 cM and 11.3 ± 0.8 cM for the PRM and WRM crosses, respectively.

^a Six 6:2, four 5:3, one 7:1 four-strand double crossover.

^b Eight 6:2, three 5:3.

^c For the PRM, the probability that chance alone could account for the excess of 4:4 segregants among the noncrossovers as compared with the crossovers (anticipated from previous studies; see text) is 0.07 (one-tailed Fisher exact test) and 0.04 (one-tailed z-test). If the 7:1 four-strand double crossover for the PRM were counted twice, the *P*-value for the Fisher exact test would be 0.05.

^d All 6:2.

FIGURE 3.—Diagram of Rine background diploids used in the *ndj1* study (Tables 11–13). The palindrome marker *HpaI-lopC* in YFS40 (*NDJ1*) and YFS41 (*ndj1*) was not scored in this study. The YFS621 strain, also used in the *ndj1* study, is diagrammed in Figure 1. YFS646 (Tables 1 and 11) is its *ndj1* derivative. Location of the DSB site is nominal on the basis of NICOLAS *et al.* (1989).

(CONRAD *et al.* 1997), reduces interference (CHUA and ROEDER 1997), and reduces noncrossover frequency (WU and BURGESS 2006). The data in CHUA and ROEDER (1997) weakly suggest an increase in crossing over.

Map lengths: To test whether an *ndj1*-induced increase in crossing over could be detected in our strains, we analyzed four-spore-viable tetrads from two sets of crosses in the Rine background (Figure 3). Table 11 indicates that the *ndj1* mutants showed an increase over wild type in all five map-length measurements, with three of the individual measurements meeting the conventional level for statistical significance. More conspicuously than the data of CHUA and ROEDER (1997), our data imply that deletion of *NDJ1* increases crossing over and may do so to a different degree in different intervals.

Interference is decreased in our *ndj1* mutant: Table 12 shows that deletion of *NDJ1* resulted in increased NPD ratios, compatible with the expected decrease in interference. While the increases for individual NPD measurements are not generally significant, all five measurements manifested the increase, while most showed significant residual interference (NPD ratio <1). For two measurements, the *m*-value (STAHL and LANDE 1995) was decreased, strengthening the interpretation of decreased interference. These data are compatible

with, but less robust than, the larger data set of CHUA and ROEDER (1997). Combined with the observed increases in crossing over, the data invite the hypothesis that deletion of *NDJ1* increases the frequency specifically of “non”-interfering crossovers at the expense of noncrossovers. Our data and calculations (described above and in the APPENDIX) suggest that noncrossovers in both the 5:3 and 6:2 classes are products primarily of the “non”-interference class of DSBs. Thus, the hypothesis predicts that an *ndj1*-induced shift from noncrossovers to crossovers should be most conspicuous in conversion tetrads.

***NDJ1* deletion decreases noncrossover and increases crossover frequencies selectively in tetrads with a conversion event at a palindrome site:** The data presented in Table 13 fulfill the expectation that the *ndj1* mutation causes a decrease in noncrossover frequency accompanied by an increase in crossovers in both the 5:3 and the 6:2 tetrads. For the 5:3 and 6:2 tetrads combined, the *ndj1* mutation reduced the noncrossover frequency by values ranging from 23 to 45% (average 31%) and increased the crossovers by values ranging from 25 to 71% (average 47%). In contrast, the changes in noncrossovers in the 4:4 class ranged from –3.3 to +2.3% (average –0.8%) and the changes in the crossover class ranged from –8.1 to +7.3% (average +1.1%) with these deviations being, for the most part, statistically insignificant despite the large numbers of tetrads in these classes. These data support the prediction that the *ndj1*-induced shift from noncrossovers to crossovers will be concentrated in conversion tetrads and suggest (1) that, within the “non”-interference class, a shift of noncrossovers to crossovers contributes to the reduced interference phenotype of the *ndj1* mutation and (2) that the 4:4 tetrads are selectively poor in “non”-interference class events.

DISCUSSION

We analyzed DSB events at hotspots marked with small palindromes that make PRMs in heteroduplex DNA and compiled the results for tetrads segregating 5:3, 6:2, or 4:4 for the palindrome. Table 14 summarizes

TABLE 11
PD/NPD/TT frequencies and map lengths in *NDJ1* and *ndj1* strains

Strain	Type	LEU-URA	MAT-KAN	MAT-NAT	KAN-NAT
YFS40	<i>NDJ1</i>	2304/6/788; 13.3 ± 0.4			—
YFS41	<i>ndj1</i>	2467/15/1009; 15.7 ± 0.5*			—
YFS621	<i>NDJ1</i>	2128/4/683; 12.6 ± 0.4	1120/84/1475; 36.9 ± 1.0	898/121/1564; 44.3 ± 1.2	2108/2/444; 8.9 ± 0.4
YFS646	<i>ndj1</i>	884/6/281; 13.5 ± 0.9	461/52/647; 41.3 ± 1.8*	369/84/676; 52.3 ± 2.2*	893/2/219; 10.4 ± 0.7

YFS40, 3171 four-spore viable tetrads of 3944 tetrads dissected. YFS41, 3598 four-spore viable tetrads of 4926 tetrads dissected. YFS621, 2876 four-spore viable tetrads of 4020 tetrads dissected. YFS646, 1218 four-spore viable tetrads of 2720 tetrads dissected. **P* < 0.05; two-tailed probability that the difference between this *ndj1* value and the *NDJ1* value above it could have arisen by chance alone (Stahl Lab Online Tools). Map lengths are in centimorgans.

TABLE 12
Interference as *m*; NPD ratio; and observed PD/NPD/TT in *NDJ1* and *ndj1* strains

Strain	Type	LEU-URA	MAT-KAN	KAN-NAT	MAT-NAT
YFS40	<i>NDJ1</i>	1; 0.20 ± 0.08; 2304/6/788	—	—	—
YFS41	<i>ndj1</i>	1; 0.33 ± 0.08; 2467/15/1009	—	—	—
YFS621	<i>NDJ1</i>	1,2; 0.16 ± 0.08; 2128/4/683	1; 0.46 ± 0.05; 1120/84/1475	0,1; 0.18 ± 0.13; 2108/2/444	1; 0.49 ± 0.05; 898/121/1564
YFS646	<i>ndj1</i>	0,1; 0.59 ± 0.24; 884/6/281	1; 0.63 ± 0.10; 461/52/647	0,1; 0.32 ± 0.23; 893/2/219	0; 0.81 ± 0.11; 369/84/676

See Table 6.

our conclusion that the 5:3 and 4:4 tetrads, for both crossover and noncrossover tetrads, have complementary features. Nonconversion (4:4) crossovers, which, as a class, are Msh4 dependent (Tables 5 and 7), display positive interference (Table 4) and promote Msh4-facilitated disjunction of homologs (ROSS-MACDONALD and ROEDER 1994). Moreover, among 4:4 tetrads the absolute frequencies of both crossovers and noncrossovers were conspicuously affected by *msh4* (Table 7), but only minimally by *ndj1* (Table 13), a gene required for normal homolog pairing (CONRAD *et al.* 1997). In

contrast, among tetrads segregating 5:3 for the palindrome site, the crossovers lacked positive interference in wild-type meioses (Table 4). Among 5:3 tetrads, the frequencies of both crossovers and noncrossovers were conspicuously affected by *ndj1* (Table 13), but only minimally by *msh4* (Table 7, Petes).

Our results suggest that the 4:4 and 5:3 segregation classes represent two classes of meiotic DSBs, each with its own rules for repair of PRMs (see the Introduction) and each yielding both crossovers and noncrossovers. Tetrads segregating 6:2 appear to include crossovers

TABLE 13
ndj1-induced changes in percentage (and number) of crossovers and noncrossovers for intervals containing *ARG4* or *HIS4* among tetrads with 5:3, 6:2, or normal 4:4 segregation for the relevant palindrome site

		5:3		6:2		5:3 + 6:2		Normal 4:4		Total
		CO	NCO	CO	NCO	CO	NCO	CO	NCO	
A. <i>HIS4</i>										
YFS621	<i>NDJ1</i>	1.5 (38)	2.2 (55)	0.9 (24)	0.7 (18)	2.4 (62)	2.9 (73)	15.0 (384)	79.7 (2035)	2554
YFS646	<i>ndj1</i>	2.8 (31)	1.3 (15)	1.3 (15)	0.3 (3)	4.1 (46)	1.6 (18)	15.6 (173)	78.7 (875)	1112
Change		+1.3	−0.9	+0.4	−0.4	+1.7	−1.3	+0.6	−1.0	
% change		+86	−41	+44	−57	+71	−45	+4.0	−1.3	
<i>P</i> ^a		0.004	0.05	0.13	—	0.003	0.01	0.34	0.25	
<i>P</i> ^b		0.006		0.1		0.001		0.68		
B. <i>ARG4</i>										
YFS40	<i>NDJ1</i>	1.3 (41)	2.2 (68)	2.2 (69)	1.8 (57)	3.6 (110)	4.0 (125)	22.0 (680)	70.4 (2178)	3093
YFS41	<i>ndj1</i>	2.0 (68)	1.4 (47)	3.3 (114)	1.6 (57)	5.2 (182)	3.0 (104)	23.6 (820)	68.1 (2362)	3468
Change		+0.7	−0.8	+1.1	−0.2	+1.6	−1.0	+1.6	−2.3	
% change		+54	−36	+50	−11	+44	−25	+7.3	−3.3	
<i>P</i> ^a		0.02	0.005	0.005	0.27	0.0005	0.01	0.06	0.02	
<i>P</i> ^b		0.002		0.05		0.0002		0.08		
C. <i>ARG4</i>										
YFS621	<i>NDJ1</i>	2.6 (72)	4.8 (135)	3.3 (93)	2.1 (60)	5.9 (165)	6.9 (195)	18.5 (522)	68.7 (1933)	2815
YFS646	<i>ndj1</i>	2.7 (32)	3.7 (43)	4.7 (55)	1.6 (19)	7.4 (87)	5.3 (62)	17.0 (198)	70.3 (821)	1168
Change		+0.1	−1.1	+1.4	−0.5	+1.5	−1.6	−1.5	+1.6	
% change		+3.8	−23	+42	−24	+25	−23	−8.1	+2.3	
<i>P</i> ^a		0.37	0.06	0.02	0.15	0.03	0.03	—	—	
<i>P</i> ^b		0.3		0.06		0.01		0.24		

CO, crossover; NCO, noncrossover.

^a One-tailed probabilities associated with the z-values calculated from the differences in the proportions of the *NDJ1* and *ndj1* classes to their respective totals.

^b Chi-square probabilities that the ratios of crossovers to noncrossovers in the *NDJ1* and *ndj1* samples could differ as much by chance alone.

TABLE 14
Properties of segregation classes for PRMs

	4:4	5:3	6:2
COs ^a have possible interference	Yes	No	Yes
CO frequency reduced by <i>msh4</i> mutation	Yes	No ^b	Yes
NCO ^c frequency increased by <i>msh4</i> mutation	Yes	No ^b	No ^d
CO frequency increased by <i>ndj1</i> mutation	No ^b	Yes	Yes
NCO frequency decreased by <i>ndj1</i> mutation	No ^b	Yes	Yes

^a Crossovers.
^b Small change?
^c Noncrossover.
^d As noted in Petes background.

from both classes (Table 7), but noncrossovers from the Msh4-independent class only (APPENDIX). The data further suggest that the DSB_r class represented by 5:3's promotes pairing but is not required for normal disjunction in wild-type crosses while the other, represented by 4:4's, promotes meiosis I disjunction and plays no conspicuous role in pairing. Henceforth, we shall refer to these two classes as "phases" of DSB_r involved in "pairing" and "disjunction," respectively.

Previously, the hypothesis of two DSB_r classes in yeast relied on statistical analysis of interference and on inference based on the phenotypes of mutants that reduce crossing over. The demonstration that, in wild-type yeast, 5:3 segregants are identifiable as products of the pairing phase confirms the validity of the hypothesis.

We note that the interference data (Tables 4 and 6) and the *msh4* data of Table 7 are variable with respect to strain and locus, as expected in the presence of two classes of DSB_r that vary in relative frequency. The entries that fail to pass statistical tests of significance often come close to doing so and never manifest opposite trends.

Phenotypes of *msh4* deletion: The use of markers making PRMs revealed new *msh4* phenotypes. Our data show, first, that among 5:3 tetrads crossover frequency is almost independent of Msh4 (Table 7, Petes) and that these Msh4-independent 5:3 crossovers lack positive interference (Table 4). This implies that inferences derived from studies involving 5:3 tetrads or heteroduplex DNA may apply only to the pairing phase of DSB_r. Second, as anticipated from an Msh4-Msh5-dependent stabilization of Holliday junctions (SNOWDEN *et al.* 2004 and see ROSS-MACDONALD and ROEDER 1994), the tetrads segregating 4:4 for the palindrome show a *msh4*-induced decrease in crossovers accompanied by an increase in noncrossovers. However, among the 6:2 tetrads, the *msh4*-induced loss of crossovers is not accompanied by an increase in 6:2 noncrossovers. Instead, the 6:2 crossovers appear to have been transformed into 4:4 noncrossovers (Table 7, Petes). This suggests that whenever Msh4 is absent or unavailable a disjunction-phase DSB is repaired as a noncrossover with 4:4 segregation for the palin-

FIGURE 4.—A model for noncrossover production via single-end invasion with synthesis-dependent strand-annealing (HABER 2000; HUNTER and KLECKNER 2001; HOFFMANN and BORTS 2004) in the disjunction phase of DSB_r. Noncrossover products arise when the invasion is not stabilized by Msh4/5, either because the meiosis is occurring in a *msh4/5* mutant or because the "interference machinery" has deprived the intermediate of Msh4/5. When a DSB is marked with a PRM on the left of the DSB, the rules dictate that MMR at invasion will fail in the disjunction phase. DNA synthesis is followed by withdrawal and capture of the other DSB fragment. Round two of MMR, mandated by the rules, then restores the normal 4:4 ratio at any PRM to the right of the DSB. This proposal is in harmony with the view (reviewed in BISHOP and ZICKLER 2004) that the double Holliday-junction precursors to interfering crossovers yield no noncrossovers, and with the view (APPENDIX) that all conversion noncrossovers are products of the pairing phase.

drome. The abundance in *MSH4* crosses of tetrads with MMR-related 4:4 segregation of the palindrome site (Table 10) suggests that these products reflect the rules for MMR in the wild-type disjunction phase. Moreover, the overrepresentation of noncrossovers among the tetrads that appear to have MMR-dependent 4:4 segregation suggests that such noncrossovers result from a programmed, interference-related lack of access to Msh4 (STAHL *et al.* 2004). In Figure 4, we offer a scenario in which noncrossovers in the disjunction phase inevitably segregate 4:4 for a marker making PRMs. In the APPENDIX, we support the view that all the *visible* (*i.e.*, conversion) noncrossovers derive from the pairing phase.

It was suggested to us, as an alternative interpretation of our data, that palindromes are prone to failing, in some situations, to enter a heteroduplex state. However, the observation (HOFFMANN *et al.* 2005) that strains compromised for MMR by *mlh1* or *msh2* mutation give increased frequencies of one-sided conversions with point mutations challenges that view.

Phenotypes of *ndj1* deletion: Our data and those of WU and BURGESS (2006) show an *ndj1*-induced reduction in noncrossovers. In our experiments, but not in those of WU and BURGESS (2006), the reduction in noncrossovers is matched with an *ndj1*-induced increase in crossovers. The difference between these two sets of results may reflect strain or locus differences or differences inherent in the methods used for analysis. For example, WU and BURGESS (2006) looked for *ndj1* phenotypes in DNA isolated from meiotic cells whereas we examined tetrads with four viable spores. Our ability to identify the classes of tetrads in which these phenotypes are concentrated secured our conclusions.

The *ndj1* phenotypes observed in our crosses—reduction in noncrossovers, increase in crossovers—characterized the conversion tetrads in which the noncrossovers are assignable to the pairing phase (APPENDIX). We propose that the observed *ndj1*-induced increase in crossovers represents an increase specifically in “non”-interfering, pairing-phase crossovers at the expense of pairing-phase noncrossovers. This increase, we propose, is responsible for the modest reduction in interference observed in our *ndj1* mutants (Table 12). CHUA and ROEDER (1997) reported a more conspicuous reduction in interference and a weaker increase in crossing over. Their *ndj1* strain differed as well in showing the classical nondisjunction phenotype of a conspicuous increase in two-spore viable tetrads (CHUA and ROEDER 1997), a phenotype not evident in our strain (supplemental Figure S1). CHUA and ROEDER (1997) also reported an increase in chromosomes that lacked crossing over (E_0 tetrads), a reasonable phenotype for pairing-defective *ndj1* mutants. We question the conventional interpretation (*e.g.*, TRELLES-STICKEN *et al.* 2000) that the increased E_0 class seen by CHUA and ROEDER (1997) is a result of diminished interference. It appears to us more likely that the increased E_0 class in their strains arises from an occasional failure of effective pairing. Such pairing failures in the *ndj1* strain of CHUA and ROEDER (1997) would account simultaneously for the greater reduction in interference and the smaller increases in crossing over by increasing the PD tetrads without imposing any changes in the frequencies of TTs and NPDs relative to each other. Pairing failures might also account for the lack of increase in crossover DNA in the studies of WU and BURGESS (2006).

Our evidence for the noncrossover-promoting role of *NDJ1* may reflect a selective advantage of noncrossover over crossover resolution in the pairing phase of DSB_r, as previously suggested by SMITHIES and POWERS (1986) and CARPENTER (1987). One may speculate that a reduction or delay in the DSB-dependent phase of chromosome pairing increases crossing over by reducing the effectiveness of an unidentified process that favors noncrossover resolution in the pairing pathway, designed to prevent translocations caused by ectopic alliances. ROCKMILL *et al.* (1995) remarked that short

chromosomes are slow to pair. Thus, the higher densities of “non”-interfering crossovers associated with shorter chromosomes (KABACK *et al.* 1999; STAHL *et al.* 2004; but see TURNEY *et al.* 2004) and with deletion of *NDJ1* may be a common consequence of slow pairing.

Noncrossovers in two phases: BÖRNER *et al.* (2004) describe a view in which an “early” noncrossover pathway of DSB_r (which also produces some, presumably noninterfering, crossovers) is the only source of noncrossovers. This view implies that these “early” noncrossovers had been programmed to be resolved as such by the interference apparatus. BÖRNER *et al.*’s (2004) description of a “noncrossover pathway” yielding both noncrossovers and some noninterfering crossovers fits our “pairing phase.” However, our experiments with PRMs suggest (1) that neither the crossovers nor the noncrossovers in this phase were affected by the interference apparatus and (2) that the disjunction phase, as well as the pairing phase, generates both noncrossovers and crossovers. Specifically, both crossovers and noncrossovers in the pairing phase, represented by the 5:3 tetrads, are responsive to the pairing-promoting *Ndj1* function but not appreciably so to the interference-promoting *Msh4* function. Conversely, noncrossovers as well as crossovers in the disjunction phase, represented by the 4:4 tetrads, are characterized by their greater responsiveness to *Msh4* than to *Ndj1* (Table 14).

Further support for the concept of two kinds of meiotic noncrossovers comes from a study of crossover homeostasis (MARTINI *et al.* 2006). Those authors suggested that some, but not all, DSBs ordinarily destined to give rise to noncrossovers gave rise to interfering crossovers under conditions of DSB shortage, leading them to propose that some DSBs may be unavailable for homeostasis. We suggest that the unavailable DSBs are, in fact, precursors to the noncrossover products of the pairing phase, while the incipient noncrossovers available for crossover homeostasis belong to the disjunction phase.

Unless DSB_r events are monitored with a marker making WRMs, as in Table 10, the use of PRMs allows no distinction between 4:4 MMR-related noncrossover tetrads and 4:4 tetrads lacking a DSB_r event. This problem may account for the view, adopted, for example, by BÖRNER *et al.* (2004), BISHOP and ZICKLER (2004), and WU and BURGESS (2006), that the pathway that generates interfering crossovers fails to generate noncrossovers. We do not dispute the view that double-Holliday-junction intermediates give rise only to interfering crossovers as suggested by ALLERS and LICHTEN (2001). However, as indicated above, we propose that the intermediates destined by the interference apparatus to be resolved as noncrossovers generate only 4:4 (*i.e.*, invisible) disjunction-phase noncrossovers when monitored with a PRM (see Figure 4), while the observed 5:3 noncrossovers (or heteroduplex DNA restriction fragments) are all products of the pairing

phase. Implied in this proposal is the notion that the interference apparatus operates after DSB-dependent pairing has been initiated.

Negative interference between pairing-phase conversions and disjunction-phase crossovers? In wild-type (*MSH4*) crosses of the Rine strain (Table 4), events in the pairing phase of DSB_r manifested (an almost statistically significant) negative interference. The map length of the *MAT-KAN* interval in the total data is 36.7 ± 1.2 cM, while the value for the combined 5:3 and 6:2 noncrossovers is 50.0 ± 8.6 cM and that for the 5:3 crossovers is 52.7 ± 14.7 cM. The *MAT-KAN* map length for those crossover and noncrossover data combined is 50.9 ± 7.6 cM. We can test whether this indication of negative interference arises from above-average cell-wide rates of crossing over in these selected tetrads. For the Rine strain, among the tetrads with 5:3 segregation, plus the noncrossover tetrads with 6:2 segregation for the palindrome site at *HIS4* (on chromosome III), the *LEU-URA* interval (on chromosome VIII) is 12.1 ± 1.9 cM as compared with 12.6 ± 0.5 cM among total tetrads. For the Petes strain, the analogous values for the *TRP-URA* interval are 18.9 ± 1.5 cM and 17.5 ± 0.6 cM, respectively. Thus, the negative interference that seems to characterize DSB_r events in the pairing pathway is localized to the chromosome on which the event occurs.

Data for the *HYG-KAN* interval (Petes background) are too few to stand on their own but manifest leanings of the same sort. In brief, the combined 5:3 crossovers and conversion noncrossovers in the *KAN-NAT* interval have a *HYG-KAN* distance of 6.1 ± 1.6 cM, as compared with the *HYG-KAN* map length in the unselected data of 4.9 ± 0.3 cM.

Because the PERKINS (1949) formula underestimates longer distances, we suspect the apparent negative interference is not a reflection of statistical inadequacy of the data. Since negative interference has not been reported for *msh4* mutant crosses, we propose that the negative interference observed in our *MSH4* crosses occurred between disjunction phase crossovers and pairing-phase conversion events.

If the negative interference is localized to the vicinity of pairing-phase events, which seems likely, it might have a corollary in cytological observations. Connections between homologs, called “axial associations” (ROCKMILL *et al.* 1995), may be visible manifestations of DSB_r events of the pairing phase. These associations appear to correlate spatially with concentrations of recombination proteins whose activities are associated with crossing over in the disjunction phase (reviewed in BISHOP and ZICKLER 2004). The possibility of physical association between events in the two phases is further supported by the studies of TSUBOUCHI *et al.* (2006). Negative interference between conversion noncrossovers and nearby crossovers might also account for recombination events in which a conversion is separated from its apparently “associated” crossover by a stretch of

unconverted markers (*e.g.*, SYMINGTON and PETES 1988; JESSOP *et al.* 2005). Such negative interference could also account for *trans* events associated with crossovers as reported by HOFFMANN and BORTS (2005).

Testing the rules: JESSOP *et al.* (2005) reported a large fraction of one-sided conversions—conversions for a marker making PRMs on one side of a DSB accompanied by 4:4 segregation at a PRM that is 300 bp on the other side. The one-sided 6:2 tetrads obeyed the rules very nicely: junction-directed MMR fully converted one mismatch while, apparently, restoring the other (see below). However, some one-sidedness was seen for 5:3 conversions, too (and see GILBERTSON and STAHL 1996). Such events, by virtue of their manifest 5:3 segregation of one marker, belong to the pairing phase, which, according to the rules, is not subject to restoration. Reconciliation between these data and the rules may lie in the possibility that these tetrads as well as our MMR-independent “one-sided” conversions, such as the 5/40 observed with two WRMs (Table 14), derive predominantly from the pairing phase and reveal structural lopsidedness unique to that phase, perhaps of the sort described by ALLERS and LICHTEN (2001).

The rules, proposed to account for the observed relationships among conversion, interference, and mismatch repair in yeast, are applicable to previously puzzling data reported for *Sordaria*. KITANI (1978) conducted tetrad analyses, similar to ours, in *S. fimicola*, all of whose mismatches appear poorly repairable. Like ours, KITANI’s data demonstrated that 5:3 crossovers lacked (positive) interference. Unlike ours, however, KITANI’s 6:2 crossovers also lacked interference. A conspicuous difference between yeast and *Sordaria* lies in the patterns of non-Mendelian segregation: *Sordaria* has a relatively high ratio of aberrant 4:4 tetrads (tetrads with two spores bearing an unrepaired mismatch at the same site) to 5:3 tetrads as compared to that for yeast markers that make PRMs (reviewed in MESELSON and RADDING 1975). This difference suggests a difference in the structure of the bimolecular intermediates in the two species. As shown in Figure 1 of STAHL and FOSS (2008, this issue), *Sordaria*’s relative abundance of aberrant 4:4 tetrads, which lack interference (KITANI 1978), implies that heteroduplex regions in *Sordaria*’s pairing phase are predominantly symmetric (heteroduplex on both participating chromatids), whereas those in yeast are predominantly asymmetric (heteroduplex on only one of the two participating chromatids). If disjunction-phase intermediates differ similarly, the rules predict that, in yeast crossovers, junction-directed MMR will lead either to restoration of 4:4 segregation or to 6:2 conversion, depending on which pair of strands, whose cutting results in resolution of a given junction, directs the repair. In our experiments, where the distances between the PRMs and either junction are almost equivalent (and, perhaps, irrelevant), one pair of strands is as likely to direct the MMR as the other pair.

Hence, according to the rules, the disjunction-phase crossovers with 6:2 segregation should represent 50% of all interfering crossovers. As pointed out above, the predominance of "one-sided" 6:2 crossovers observed by JESSOP *et al.* (2005) could be the result of restoration on one side of the DSB occurring hand in hand with MMR to 6:2 on the other. If *Sordaria*, on the other hand, has predominantly symmetric heteroduplex in its disjunction phase, junction-directed repair of PRMs will lead to restoration only (STAHL and HILLERS 2000), regardless of which resolved junction directs the repair. Thus, KITANI's (1978) observation that, in *Sordaria*, interference can be detected only among normal 4:4 tetrads is in complete harmony with the rules. In our data, on the other hand, the lack of interference in the 6:2 pairing-phase crossovers was masked by the interference of the 6:2 disjunction-phase crossovers derived by MMR from asymmetric heteroduplex. KITANI's 1978 article is discussed further by STAHL and FOSS (2008, this issue).

The rules also account for the differences between our data and those reported by MORTIMER and FOGEL (1974) and MALKOVA *et al.* (2004) with respect to interference among conversion crossovers. These authors used WRMs, allowing them to register most or all nearby DSB events as 6:2 conversions. They showed that, unlike our combined 5:3 and 6:2 conversion crossovers (Table 4), their conversion crossovers manifest interference. The rules suggest that the use of PRMs in our experiments caused about half of the potential interfering conversion crossovers to be lost as conversions, as a result of MMR-related restoration to 4:4 segregation. In our experiments, this loss of interfering conversion crossovers allowed the negative interference of the 5:3 crossovers and the positive interference of the remaining interfering 6:2 crossovers to cancel out when we combined those two types.

The counting model for interference: The counting model for interference (Foss *et al.* 1993), designed to describe the interference phase of DSB, predicts that the region between two close crossovers will be enriched for noncrossover conversions. That a test of this prediction (Foss and STAHL 1995) gave a contrary result may have been due, in part, to the presence of pairing-phase crossovers, not governed by the usual rules of interference. Our proposal that PRMs in noncrossovers from the disjunction phase are repaired to invisibility (4:4 segregation) suggests that an additional factor may have been in play: Foss and STAHL (1995) may have detected an enrichment of invisible, disjunction-phase noncrossover events occurring at the expense of visible, pairing-phase ones. Such interference between noncrossovers, occurring at a limited number of sites in a short interval, might account for the observed decrease in visible noncrossovers in the interval between crossovers, where the counting model predicted an increase.

We offer this commutation for the counting model notwithstanding the assertion by MARTINI *et al.* (2006,

p. 294) that their data represent "strong evidence against a 'counting' model," referring to the model of Foss *et al.* (1993). That assertion was made without acknowledging the previously offered (STAHL *et al.* 2004) explicit reconciliation between the counting model and data showing that interference is maintained even as a shortage of DSBs results in the homeostatic loss of noncrossovers in favor of crossovers. The reconciliation proposed that the elements counted, rather than being DSBs, were precursors to DSBs.

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APPENDIX: ON THE ORIGIN OF CONVERSION NONCROSSEOVERS

Table 7 shows that the crossovers and noncrossovers with 5:3 conversion (for *ARG4* or *HIS4*) were minimally sensitive to the absence of Msh4. This indicates that the 5:3 tetrads include primarily products of the “non”-interference class. Tetrads with 6:2 conversion, on the other hand, included both Msh4-dependent, interfering crossovers and Msh4-independent crossovers, as well as noncrossovers. To determine whether these 6:2 noncrossovers also included products from both classes, we assumed that MMR in the “non”-interference class operates indiscriminately on incipient crossovers and noncrossovers. This assumption is consistent with the rules, which allow only limited, invasion-directed MMR in the “non”-interference class (at which stage crossovers and noncrossovers are assumed to be not yet differentiated).

The assumption that the degree of MMR in the “non”-interference class (invasion directed, leading to 6:2) should be the same for crossovers and noncrossovers may be stated as follows: Within the “non”-interference class, the fraction of 6:2 noncrossovers among total conversion noncrossovers should equal the fraction of 6:2 crossovers among total conversion crossovers. The number of “non”-interfering conversion crossovers may be measured directly as the number of Msh4-independent crossovers. For the noncrossover conversions, on the other hand, contributions from the “non”-interference class cannot be distinguished from those of the interference class. If, however, *all* of the observed conversion noncrossovers had come from the “non”-interference class, we could write $(6:2 \text{ noncrossovers}) / (5:3 + 6:2 \text{ noncrossovers}) = (\text{Msh4-independent } 6:2 \text{ crossovers}) / (5:3 \text{ crossovers} + \text{Msh4-independent } 6:2 \text{ crossovers})$. Table A1 indicates that the equality is upheld, supporting the hypothesis that all the conversion noncrossovers are products of the “non”-interference DSB_r class (see Figure 4). This conclusion is congruent with the observation (Table 4) that 6:2 noncrossovers, like the 5:3 noncrossovers (and crossovers), appear to manifest negative interference.

TABLE A1
Origin of conversion noncrossovers

	<i>msh4</i> Petes data		<i>MSH4</i> Petes data ^a		Combined data ^b	
	Noncrossover ^c	Crossover ^d	Noncrossover ^c	Crossover ^d	Noncrossover ^c	Crossover ^d
<i>ARG4</i>	$44/(44 + 5) = 0.898$	$44/(44 + 10) = 0.815$	$78/(78 + 13) = 0.857$	$2.84/(2.84 + 0.64) = 0.816$	0.871	0.815
<i>HIS4</i>	$14/(14 + 103) = 0.120$	$16/(16 + 75) = 0.176$	$33/(33 + 153) = 0.177$	$1.24/(1.24 + 7.11) = 0.149$	0.155	0.157
Average		0.89			1.12	1.03

Calculations are based on the data of Table 7. “Combined data” refers to the combined data from the *msh4* and *MSH4* crosses.

^a Source for all values except the 6:2 Msh4-independent crossovers.

^b Combining the data deals with the slight *msh4*-induced shift of 5:3 crossovers into the 5:3 noncrossover class.

^c Fraction of noncrossover conversions that are 6:2.

^d Fraction of Msh4-independent conversion crossovers that are 6:2.