Competition Between Adjacent Meiotic Recombination Hotspots in the Yeast *Saccharomyces cerevisiae*

Qing-Qing Fan, Fei Xu, Michael A. White[1] and Thomas D. Petes

Department of Biology, Curriculum of Genetics and Molecular Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280

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ABSTRACT

In a wild-type strain of *Saccharomyces cerevisiae*, a hotspot for meiotic recombination is located upstream of the *HIS4* gene. An insertion of a 49-bp telomeric sequence into the coding region of *HIS4* strongly stimulates meiotic recombination and the local formation of meiosis-specific double-strand DNA breaks (DSBs). When strains are constructed in which both hotspots are heterozygous, hotspot activity is substantially less when the hotspots are on the same chromosome than when they are on opposite chromosomes.

CHROMOSOMAL regions with unusually high levels of recombination are termed "hotspots" (reviewed by LICHTEN and GOLDMAN 1995). In fungal systems, hotspots can be defined in several ways. First, as in other systems, hotspots are regions of the chromosome that have more crossovers than expected on the basis of the physical size of the interval. Alternatively, hotspots may represent regions in which markers exhibit an unusually high level of aberrant segregation (gene conversion and postmeiotic segregation) (reviewed by PETES *et al.* 1991). In many fungal systems, a gradient in the level of aberrant segregation (a polarity gradient) is observed from one end of the gene to the other. A hotspot can also be defined as the region located at the high end of the polarity gradient or the region of DNA responsible for generating the gradient. Hotspots are of importance in understanding meiotic recombination because these regions probably represent preferred sites for the initiation of recombination events.

In the yeast *Saccharomyces cerevisiae*, naturally occurring hotspots located near the *HIS4* (WHITE *et al.* 1991, 1993; DETLOFF *et al.* 1992), *ARG4* (NICOLAS *et al.* 1989; SUN *et al.* 1991; DE MASSY *et al.* 1993) and *HIS2* (MALONE *et al.* 1994) genes have been extensively characterized. In addition, hotspots were observed for certain insertions transformed into the yeast chromosomes including a DNA segment with *LEU2* and bacterial plasmid sequences (CAO *et al.* 1990), a Tn3-derived transposable element (STAPLETON and PETES 1991), and oligonucleotides containing telomeric sequences (WHITE *et al.* 1993; FAN *et al.* 1995).

Corresponding author: Thomas D. Petes, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.
E-mail: tompetes@email.unc.edu

[1] *Present address:* Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

The *HIS4* and *ARG4* hotspots share a number of common features (reviewed by NICOLAS and PETES, 1994). First, both are located upstream of the gene at the peak of a polarity gradient, and both stimulate recombination bidirectionally. Second, meiosis-specific double-strand DNA breaks (DSBs) colocalize to the hotspot regions at both loci (SUN *et al.*, 1989, 1991, NAG and PETES 1993), and alterations affecting hotspot activity show proportional alterations in the level of DSBs (DE MASSY and NICOLAS 1993; FAN *et al.* 1995). For both loci, the DSBs occur in regions of the chromosome that are sensitive to *in vitro* digestion with nucleases (OHTA *et al.* 1994; FAN and PETES 1996). In general, regions with high levels of DSBs represent nuclease-sensitive regions of chromatin (WU and LICHTEN 1994) but some nuclease-sensitive regions are not hotspots for DSB formation (WU and LICHTEN 1995; FAN and PETES 1996). A final common feature, as described in more detail below, is that both hotspots, when heterozygous, show disparity of gene conversion (NICOLAS *et al.* 1989; WHITE *et al.* 1991, 1993; DETLOFF *et al.* 1992).

For a heterozygous locus (alleles *A* and *a*), gene conversion events represent tetrads in which the markers segregate 3*A*:1*a* or 1*A*:3*a* instead of the expected Mendelian ratio of 2*A*:2*a*. Although there are only four spores per ascus in *S. cerevisiae*, we will use the nomenclature derived from eight-spored asci in which conversion tetrads represent 6*A*:2*a* or 2*A*:6*a* for the sake of consistency with other fungal genetic papers. In most experiments, the two types of gene conversion events are observed with equal frequencies (parity). Disparity of conversion is observed in strains in which the scored markers represent heterozygous hotspots or are closely linked to heterozygous hotspots. If *A* represents a functional hotspot and *a* the nonfunctional hotspot, the observed disparity is that the 6*A*:2*a* class of tetrads is significantly smaller than the 2*A*:6*a*. This result indicates that the chromosome that initiates recombination

(the chromosome with the hotspot) functions as a recipient of information rather than the donor (reviewed by PETES *et al.* 1991). This observation was important in the development of the double-strand break repair model of recombination (SZOSTAK *et al.* 1983). In this model, recombination initiates by a double-strand DNA break with the broken ends invading the unbroken chromosome. Subsequent heteroduplex formation, followed by mismatch repair, results in information transfer from the unbroken chromosome to the chromosome with the initiating lesion.

Although there is a clear correlation between hotspot activity and DSB formation, why certain sites are preferred targets of DSB formation is still not fully understood. One possibility is that hotspots simply represent open regions of chromatin accessible to the nuclease that initiates DSB formation. Alternatively, DSB formation may require interactions between the nuclease and various DNA binding proteins. This possibility is suggested by the observation the natural hotspot upstream of *HIS4* contains binding sites for four transcription factors (Gcn4, Bas1p, Bas2p, and Rap1p), and all of these proteins, except Gcn4p, are required for hotspot activity (WHITE *et al.* 1991, 1993) and DSB formation (FAN *et al.* 1995). Although transcription factors are required for *HIS4* hotspot activity, a high level of transcription is not, since a deletion of the *HIS4* TATAA element, which reduces transcription about a factor of 20, has no effect on recombination (WHITE *et al.* 1992).

In strains with the wild-type hotspot, the frequency of aberrant segregation of markers within *HIS4* varies from 50% at the 5′ end to 15% at the 3′ end (DETLOFF *et al.* 1991, 1992). Deletion of the hotspot eliminates this gradient resulting in a level of ~15% aberrant segregation for markers at all positions within the gene. A 51-bp region of telomeric DNA substituting for the wild-type hotspot results in an extremely strong hotspot (WHITE *et al.* 1993) and a very high level of DSBs (FAN *et al.* 1995). The telomeric sequence contains multiple binding sites for Rap1p. The hotspot-generating activity of the telomeric sequence may be related to its ability to bind Rap1p, since other oligonucleotides with multiple Rap1p binding sites strongly stimulate exchange (WHITE *et al.* 1993).

When sequences with hotspot activity are reinserted into various chromosomal regions, the activity of the hotspot varies with the chromosome context (WU and LICHTEN 1995). One observation related to context effects is that a high frequency of DSBs at one site suppresses DSBs at a nearby site. Three different studies involving different hotspots have detected such interactions (FAN *et al.* 1995; WU and LICHTEN 1995; XU and KLECKNER 1995). In this paper, we analyze interactions between two closely linked hotspots, the wild-type hotspot located near the 5′ end of the *HIS4* gene and a hotspot resulting from insertion of telomeric sequences into the coding region of *HIS4*. We present genetic and physical data indicating that the activity of the hotspots is reduced when they are located on the same chromosome.

MATERIALS AND METHODS

Plasmids: A number of different plasmids were used to make alterations in the region upstream of *HIS4* or in the *HIS4* coding sequences. The plasmid pPD5 has a *Sau*3A fragment of the *HIS4* promoter region with the deletion mutation *his4-Δ52* inserted into B142, a derivative of YIp5 that lacks the *Pvu*II site (WHITE *et al.* 1993); the region deleted in *his4-Δ52* (−114 to −316) contains the wild-type *HIS4* recombination hotspot (DETLOFF *et al.* 1992). The plasmid AB457 (provided by K. ARNDT, Cold Spring Harbor Laboratory) has a *Sau*3A fragment containing the promoter region of *HIS4* gene inserted into the *Bam*HI site of YIp5 with a 31-bp deletion (from −132 to −101, *HIS4-445*) that removes the TATAA sequences. The plasmid pC1G17 (provided by L. SYMINGTON, Columbia University) has a *Bam*HI fragment containing the *HIS4* gene with a "fill-in" of the *Sal*I site at the 5′ end of the gene, generating the *his4-Sal* mutation (WHITE *et al.* 1992). The plasmid pSH17 (provided by S. HENDERSON) contains an oligonucleotide with the telomeric sequences inserted into the *Xho*I site of a *Sal*I-*Sph*I fragment containing the *HIS4* gene (*his4-203*). The sequence of one strand of the oligonucleotide was: 5′ TCGACTGCAGAGTGGGTGTGGTGTGGTGTGGGT-TGGTGTGTGTGTGGGTGGTGGTGTGGGACAGCTG 3′. The complementary strand was designed to leave 5′ protruding TCGA sequences, compatible with the ends generated by *Xho*I.

The plasmid AB328 (provided by K. ARNDT) contains a yeast DNA fragment with the *bas2-2* mutation inserted into YIp5. The plasmid pNKY349 (CAO *et al.* 1991) was used to construct strains with the *rad50S* mutation. The plasmid pDN42, which was used as hybridization probe in several experiments, has a 1.6-kb *Xho*I-*Bgl*II fragment that includes most of the *HIS4* coding sequence inserted into *Xho*I-*Bam*HI-treated Bluescript pBSIISK(−) (NAG and PETES 1993).

Yeast strains: All haploid yeast strains are isogenic with either AS4 (*alpha trp1 arg4 tyr7 ade6 ura3*) or AS13 (*a leu2 ade6 ura3*) except for mutations introduced by transformation. All constructions were performed by two-step transplacement (ROTHSTEIN 1983), except for those of the *rad50S* strains which were performed by one-step transplacement with pNKY349. The haploid strain DNY106 is a *rad50S* derivative of AS13. The construction of other haploid strains used in this study is summarized in Table 1.

Isogenic pairs of *RAD50* and *rad50S* diploid strains were constructed by mating the following haploids (*RAD50* strains listed first): (1) SH106 (SH20 × AS13), QFY119 (QFY31 × DNY106); (2) MW155 (MW74 × PD57), QFY129 (QFY45 × QFY46); (3) MW157 (MW76 × MW62), QFY114 (QFY23 × QFY24); (4) QFY116 (QFY25 × QFY26), QFY117 (QFY27 × QFY28); (5) PD15 (PD12 × PD13), QFY118 (QFY29 × QFY30); (6) QFY125 (SH20 × PD57), QFY127 (QFY46 × QFY31); (7) QFY126 (MW74 × AS13), QFY128 (QFY45 × DNY106).

Strains DBY939 (*alpha suc2-215 ade2-101*) and SS-4A (*a SUC4 ade1*) were used in constructing diploids used to make strains for allelism tests. Tester strains QFY40 (*a ade2 his4-Δ52*) and QFY41 (*alpha ade2 his4-Δ52*) were selected from dissected spores of QFY123 (DBY939 × PD57). Tester strains QFY42 (*a ade1 his4-203*) and QFY43 (*alpha ade1 his4-203*) were selected from dissected spores of QFY124 (SH20 × SS-4A).

Media and genetic techniques: Standard methods were used for mating transformation, and dissection (STAPLETON

TABLE 1

Construction of related haploid strains

Name	Upstream alteration	Alterations of coding sequence	Other changes	Parental strain	Plasmid used to insert alteration	Name of *rad50s* derivative
SH20	WT	*his4-203*	WT	AS4	pSH17	QFY31
MW74	*his4-Δ52*	*his4-203*	WT	SH20	pPD5	QFY45
MW71	WT	WT	*bas2-2*	AS4	AB328	QFY13
MW76	WT	*his4-203*	*bas2-2*	MW71	pSH17	QFY23
QFY25	*his4-445*	*his4-203*	WT	SH20	AB457	QF727
PD12	*his4-445*	WT	WT	AS4	AB457	QFY29
PD57	*his4-Δ52*	WT	WT	AS13	pPD5	QFY46
MW62	WT	WT	*bas2-2*	AS13	AB328	QFY24
QFY26	*his4-445*	WT	WT	AS13	AB457	QFY28
MW1	WT	*his4-Sal*	WT	AS13	pC1G17	
PD13	*his4-445*	*his4-Sal*	WT	MW1	AB457	QFY30

and PETES 1991). To score the two different *his4* alleles segregating in spores derived from strains QFY125 and strain QFY126, we used standard allelism tests as described by DETLOFF *et al.* (1992). In brief, replicas of each spore colony were mated to tester strains (described above) that contained either *his4-203* or *his4-Δ52* mutations. The mated colonies were then replica-plated to medium lacking adenine in order to select diploids. Following overnight growth at 30°, these diploids were replica-plated to medium lacking histidine and treated with UV light to stimulate heteroallelic recombination. His^+ papillations (indicating heteroallelism for the allele tested) were scored after 2 days of growth at 30°.

Analysis of DSBs in DNA: Procedures to detect meiosis-specific DSBs by agarose gel electrophoresis were done as described previously (FAN *et al.* 1995). The analysis of DSBs in strain QFY114 by polyacrylamide gel electrophoresis was done as described by XU and PETES (1996). In brief, meiotic DNA was treated with *Ase*I, which cuts the *HIS4* coding sequence ~300 bp from the approximate position of the DSB sites. This DNA was denatured and the resulting fragments separated on a polyacrylamide gel. The fragments were transferred to Hybond N+ membranes and hybridized to a labeled strand-specific probe designed to detect the 3′ ends resulting from the DSBs (XU and PETES 1996).

The probe was generated by using the unidirectional polymerase chain reaction (PCR) with primer 7982 (5′ CGTATTCCTTCTTACTATTCCATGAG) and a 692-bp DNA fragment containing sequences flanking *his4-203*; this fragment was prepared by PCR of QFY114 genomic DNA using oligonucleotides 11785 (5′ TAATTGCTAAACCCATGCACAGTG) and 12506 (5′ AACATATCCTTGCTTAATTTCTT). To the DNA sample used to map the 3′ ends, we added a small amount of a size standard to allow alignment of the DSB ends with the DNA sequencing ladder. This 285-bp standard was prepared by PCR of genomic DNA of QFY114 with the primers 11785 and 7982 described above. The DNA samples representing the DNA sequencing ladder were prepared using the thermal cycle DNA sequencing kit (fmol DNA sequencing system, Promega) as described previously; the substrate for the PCR reaction was the 692-bp DNA fragment described above and the primer used was oligonucleotide 11785. To map the 3′ ends representing DSB sites, we aligned the bands in the *Ase*I-treated sample (using the internal size standard) with the appropriate site in the DNA sequencing ladder.

Statistical analysis: Statistical comparisons were done either using the chi-square test (in experiments with specific theoretical expectations) or the Fisher exact test. P values <0.05 were considered statistically significant.

RESULTS

In previous studies, we have shown that hotspot activity for the meiotic recombination hotspot located upstream of the *HIS4* gene requires binding of three transcription factors (Bas1p, Bas2p, and Rap1p) (WHITE *et al.* 1991, 1993). High levels of transcription, however, are not required for activity of this hotspot, since a deletion of the *HIS4* TATAA sequence does not reduce recombination (WHITE *et al.* 1992). We also found that an insertion of 51 bp of telomeric DNA, replacing the wild-type recombination hotspot, resulted in strong hotspot activity and a high level of meiosis-specific double-strand DNA breaks (DSBs) (WHITE *et al.* 1993; FAN *et al.* 1995). The same telomeric sequence also strongly stimulated recombination and DSB formation at the *ARG4* locus (WHITE *et al.* 1993; FAN *et al.* 1995). We have extended these studies to examine the effect of a telomeric insertion within the *HIS4* coding sequence. As described below, we find that this insertion stimulates recombination, and that this hotspot interacts with the wild-type hotspot. The interaction is a mutual suppression of hotspot activity when the hotspots are located on the same chromosome, although little or no effect is seen when the hotspots are on opposite chromosomes.

Effects of an insertion of telomere sequences within the *HIS4* coding region on meiotic recombination: We constructed a strain (SH106) in which a telomeric insertion within the *HIS4* coding sequence (*his4-203*) was heterozygous. The observed level of aberrant segregation was 37% (Table 2), similar to the previously observed rates observed in the same strain background for alleles at similar positions (31–42%, DETLOFF *et al.* 1992). In addition, no significant difference in the number of $6^+:2^-$ and $2^+:6^-$ tetrads was observed (chi-square value of 2.0, $P = 0.16$). As discussed in the Introduction, if the telomere insertion represented by *his4-203* functioned as a hotspot, one would expect significantly more $6^+:2^-$ tetrads than $2^+:6^-$ tetrads. Tetrads with $8^+:0^-$ tetrads and $0^+:8^-$ are likely to reflect two independent recombina-

TABLE 2

Patterns of aberrant segregation at *HIS4* locus and level of hotspot-associated double-strand breaks

Strain (*rad50S* derivative)	Relevant genotype		No. of tetrads in each class					Total tetrads	Percentage Ab.Seg.	DSBs[a]		His phenotype[b]
			6:2	2:6	8:0	0:8	PMS			Upstream, %	Coding, %	
SH106 [QFY119]		*his4-203* / HIS4	56	42	17	5	0	322	37	1.9–2.4	4.7–5	His^+
MW155 [QFY129]	*his4-Δ52* / his4-Δ52	*his4-203* / HIS4	53	18	16	0	3	234	38	0	8.6–8.9	His^-
MW157 [QFY114]	*bas2-2* / bas2-2	*his4-203* / HIS4	51	16	17	0	2	280	31	0	4.4	$His^\pm$
QFY116 [QFY117]	*HIS4-445* / HIS4-445	*his4-203* / HIS4	22	27	5	8	0	192	32	1.3–2.2	3.4–4.4	$His^\pm$
PD15[c] [QFY118]	*HIS4-445* / HIS4-445	*his4-Sal* / HIS4	22	43	3	4	0	294	25	2–2.5	0	$His^\pm$

[a] Samples from *rad50S* strains of various genotype were incubated for 24 hr in sporulation medium. DNA was then isolated and treated with *Bgl*II. The resulting fragments were separated by agarose gel electrophoresis and examined by Southern analysis using a *HIS4*-specific probe. The amount of radioactivity in all *Bgl*II fragment was determined. The amount of each band representing a DSB was divided by the total radioactivity in all *HIS4* specific bands and converted to a percentage. The two DSBs associated with *his4-203* were added together.

[b] The ability of the diploids to grow in medium without histidine is indicated: His^+ (wild-type growth rate), $His^\pm$ (slow growth), His^- (no growth).

[c] Genetic data from WHITE *et al.* (1992).

tion events. If the number of tetrads in the 6:2 or 8:0 segregation classes (73) is compared with the number with 2:6 or 0:8 segregation (47), we find a significant difference (chi-square value of 4.3, $P = 0.04$). In summary, the genetic analysis of SH106 suggests that the *his4-203* allele has only very weak hotspot activity.

In general, recombination hotspots in *S. cerevisiae* represent sites for the formation of DSBs (LICHTEN and GOLDMAN 1995). Strains with a *rad50S* mutation allow the accurate mapping and quantitation of DSBs because this mutation prevents processing of the DSBs into mature recombination structures (ALANI *et al.* 1990). Strains with the *rad50S* mutation and a wild-type *HIS4* hotspot have a DSB in the region between the 5′ end of *HIS4* and the 3′ end of the neighboring *BIK1* gene (FAN *et al.* 1995). About 2.6–5% of the meiotic DNA molecules have a break in this region. We constructed a *rad50S* derivative of SH106 and examined *HIS4*-associated DSBs (QFY119). Because of the weak disparity of the *his4-203* mutation, we expected ~2.6–5% DSB at the site of the wild-type hotspot and very little (or no) DSB at the site of the telomeric insertion. To our surprise, we found ~2% DSB at the site of the wild-type hotspot but 5% DSB at two sites within the *HIS4* coding sequence flanking the telomeric insertion (Table 2 and Figure 1). Breaks within the coding sequence are never found in isogenic strains lacking the *his4-203* allele (FAN *et al.* 1995). Thus, the physical data suggest that telomeric insertion represented by *his4-203* is a strong hotspot for DSB formation, whereas the genetic data indicate that the insertion is a very weak hotspot.

One interpretation of this result is that the level of DSBs does not faithfully reflect hotspot activity. An alternative possibility is that the wild-type hotspot and the hotspot caused by the telomeric insertion interact to obscure the disparity expected for a heterozygous hotspot associated with the telomere insertion. For example, if the activity of the wild-type hotspot was inhibited by the *his4-203* hotspot in *cis* but not in *trans*, then hotspot activity on the chromosome with the two partly suppressed hotspots might be balanced by the hotspot activity of the unsuppressed wild-type hotspot on the other chromosome, resulting in a lack of disparity.

To examine this issue further, we constructed a strain (MW155) that was heterozygous for *his4-203* and homozygous for *his4-Δ52*, a deletion that removes the wild-type hotspot. As shown in Table 2, in this strain, we observed a strong (three- to fourfold) gene conversion disparity for *his4-203*, indicating that the telomere insertion in *HIS4* has hotspot activity. In addition, the aberrant segregation frequency of *his4-203* (38%) is considerably higher than the aberrant segregation frequencies of other *his4* mutant alleles in strains homozygous for *his4-Δ52* (11–21%; DETLOFF *et al.* 1992). In the *rad50S* derivative of MW155 (QFY129), no DSB was observed in the region upstream of *HIS4*, as expected, and two strong DSBs (9% of the intact *HIS4* fragment) were seen near the telomeric insertion (Figure 1).

These results demonstrate that *his4-203* results in gene conversion disparity, as expected for a heterozygous hotspot, but that this disparity is only observed in strains with the *his4-Δ52* mutation. The *his4-Δ52*

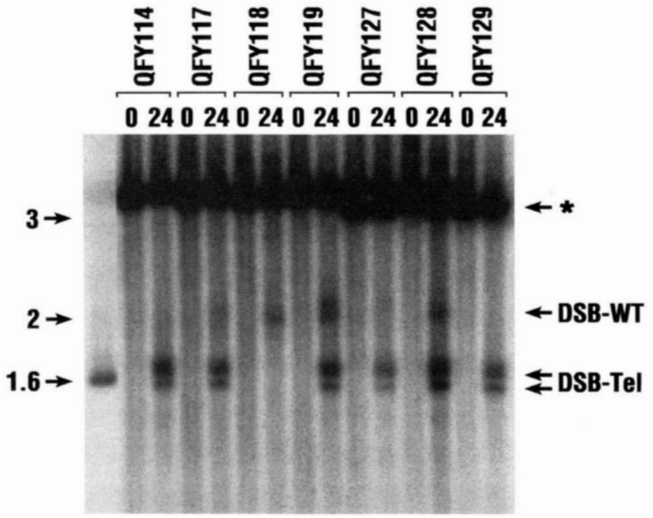


FIGURE 1.—Southern analysis of meiosis-specific DSBs at the *HIS4* locus in strains with various combinations of hotspot sequences. Cells were harvested after 0 or 24 hr in sporulation medium. DNA was isolated, treated with *Bgl*II, and the resulting DNA fragments were separated by agarose gel electrophoresis. The plasmid pDN42 (described in MATERIALS AND METHODS) was used as a *HIS4*-specific hybridization probe. Each strain is represented by two lanes and numbers above the lanes indicate the time in sporulation medium. The arrow marked by an asterisk shows the position of intact *Bgl*II fragments; strains with the *his4-Δ52* mutation have a *Bgl*II fragment ~150 bp smaller than wild-type strains. The arrow marked as DSB-WT shows the position of DSBs at the wild-type hotspot located upstream of *HIS4*. The arrows marked as DSB-Tel indicate the position of the DSBs at the site of *his4-203*, a mutant allele created by insertion of telomeric sequences. The arrows on the left side show the positions of molecular markers with sizes indicated in kb. Relevant genotypes of the strains are: QFY114 (*his4-203/HIS4 bas2-2/bas2-2*), QFY117 (*HIS4-445/HIS4-445 his4-203/HIS4*), QFY118 (*HIS4-445/HIS4-445 his4-Sal/HIS4*), QFY119 (*his4-203/HIS4*), QFY127 (*HIS4/his4-Δ52 his4-203/HIS4*), QFY128 (*his4-Δ52/HIS4 his4–203/HIS4*), and QFY129 (*his4-Δ52/his4-Δ52 his4-203/HIS4*).

mutation could have two types of effects on the ability of *his4-203* to function as a hotspot. First, as described above, the region removed by the *his4-Δ52* deletion contains the wild-type hotspot. If two adjacent hotspots suppress each other, the *his4-Δ52* deletion would remove this suppression. Second, the *his4-Δ52* deletion also removes binding sites for transcription factors required for *HIS4* expression. If transcription across *his4-203* in a strain with a wild-type promoter eliminates its hotspot activity, the *his4-Δ52* deletion might activate the *his4-203* hotspot by eliminating this transcription. It has been shown previously that transcription across the *ARG4* hotspot reduced its activity (ROCCO *et al.* 1992).

The first of these alternatives is more likely since we observe a DSB near the *his4-203* insertion in QFY119, indicating that at this hotspot, unlike the *ARG4* hotspot, DSB formation is not eliminated by transcription (Figure 1). To examine this issue in more detail, we constructed strains that were heterozygous for *his4-203* and homozygous for either *bas2-2* (MW157) or *HIS4-445* (QFY116) mutations. Binding of the Bas2p to a site in front of *HIS4* stimulates transcription ~10-fold (ARNDT *et al.* 1987; TICE-BALDWIN *et al.* 1989), and this binding is also required for the wild-type hotspot activity (WHITE *et al.* 1993). The *bas2* mutation eliminates the DSB that normally occurs in the upstream region (FAN *et al.* 1995 and Figure 1). The *HIS4-445* mutation deletes the TATAA sequence required for normal rates of *HIS4* transcription, reducing transcription ≥20-fold (NAGAWA and FINK 1985; WHITE *et al.* 1992). Strains with the *HIS4-445* deletion (such as PD15 in Table 2) have a wild-type level of aberrant segregation (WHITE *et al.* 1992) and a *rad50S* derivative of PD15 (QFY118) has a wild-type level of DSB in the upstream region (Table 2, Figure 1).

As shown in Table 2, the *his4-203* mutation in MW157 (*bas2* strain) shows the same disparity as observed in MW155 (*his4-Δ52* strain), whereas no disparity is observed in QFY116 (*HIS4-445* strain). Thus, although the level of transcription is lower in QFY116 than in MW157, there is no effect on gene conversion disparity in QFY116. The simplest interpretation of this result is that hotspot activity of *his4-203* is affected by adjacent hotspot activity rather than by the rate of transcription across the hotspot sequence.

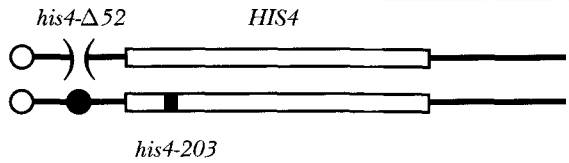
This conclusion does not rule out a small effect of transcription on hotspot activity. In particular, the level of DSBs observed in MW157 is somewhat reduced from that observed in MW155. In addition, the hotspot activity in MW157 (31% aberrant segregation), which has a reduced level of *HIS4* transcription, is slightly weaker than that observed in MW155 (38% aberrant segregation), which has no *HIS4* transcription. This difference, however, is not statistically significant ($P = 0.08$ by Fisher exact test).

In summary, the level of gene conversion in SH106 reflects a complicated interaction between the wild-type hotspot located upstream of *HIS4* and the *his4-203* hotspot within the gene. We suggest that, in the chromosome with two hotspots, there is a suppression of the activities of both hotspots. The net hotspot activity of these suppressed hotspots is approximately equivalent to that observed for the single wild-type hotspot on the opposite chromosome. Thus, we observe no conversion disparity in SH106. To provide further evidence for this conclusion, we examined recombination in strains heterozygous for both *his4-203* and the wild-type hotspot.

Competition between recombination hotspots located in *cis* or in *trans*: Two strains heterozygous for both the wild-type and the *his4-203* hotspots were constructed. In strains QFY125 and QFY126, the hotspots were arranged in *cis* and in *trans*, respectively. The genetic data are shown in Tables 3 and 4, and summarized in Figure 2. As shown in Figure 2, the level of aberrant segregation for *his4-Δ52* is elevated from 17% in QFY125 to 28% in QFY126. Similarly, the aberrant seg-

TABLE 3

Number of tetrads in various classes of aberrant segregants for *his4* mutant alleles in QFY125



his4-203 / his4-Δ52	6+:2m	2+:6m	8+:0m	0+:8m	4+:4m	Totals
6+:2m	0	8	0	0	8	16
2+:6m	26	0	1	0	10	37
8+:0m	0	0	0	0	0	0
0+:8m	0	0	1	0	0	1
4+:4m	26	12	3	0	221	262
Totals	52	20	5	0	239	316

The positions of the wild-type and *his4-203* hot spots are indicated by the solid circle and bar, respectively.

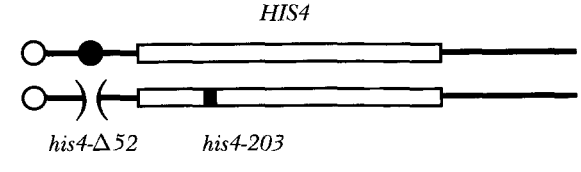
regation rate of *his4-203* is elevated from 24 to 36%. Statistical comparisons (Fisher's exact test) of the number of tetrads that show aberrant segregation for these two loci in the two different strains indicate that these differences are significant (P values <0.01 for both comparisons). These data indicate that the hotspots promote recombination more effectively when they are located on opposite chromosomes than when they are on the same chromosome.

As discussed in the Introduction, heterozygous hotspots usually show disparities in the ratio of conversion events. Since the levels of aberrant segregation at each hotspot represent a sum of events initiated at that hotspot plus events propagated from initiation events at the other hotspot (coconversion events), the level of disparity for individual hotspots is best estimated by examining only those events in which one hotspot undergoes conversion and the other segregates 4:4. In QFY125 (*cis* arrangement of hotspots), *his4-203* shows modest disparity (26 6:2 tetrads and 12 2:6 tetrads) in tetrads in which the *his4-Δ52* allele has normal Mendelian segregation, indicating modest hotspot activity when located in *cis* to *his4-Δ52*. In the same strain, the *his4-Δ52* allele has no significant disparity (8 6:2 tetrads and 10 2:6 tetrads), indicating that the wild-type hotspot has no detectable activity when located *cis* to *his4-203*. In contrast, in QFY126 (*trans* arrangement of hotspots), both hotspots show significant disparity of conversion in the expected direction.

The hotspots also promote crossovers between *HIS4* and *LEU2* more effectively in the *trans* than in the *cis* configuration. In QFY125, in tetrads in which *his4-203*, *his4-Δ52*, and *LEU2* were showing normal Mendelian segregation, the distance between *LEU2* and *his4-203* was 13.6 cM (156 parental ditype and 58 tetratype tetrads). In QFY126, the map distance between the same markers was 22.3 cM (95 parental ditype and 77 tetratype tetrads). The difference in the proportion of

TABLE 4

Number of tetrads in various classes of aberrant segregants for *his4* mutant alleles in QFY126



his4-203 / his4-Δ52	6+:2m	2+:6m	8+:0m	0+:8m	4+:4m	Totals
6+:2m	21	0	3	0	10	34
2+:6m	5	22	1	1	22	51
8+:0m	0	0	0	0	0	0
0+:8m	1	1	0	2	1	5
4+:4m	43	5	10	0	169	227
Totals	70	28	14	3	202	317

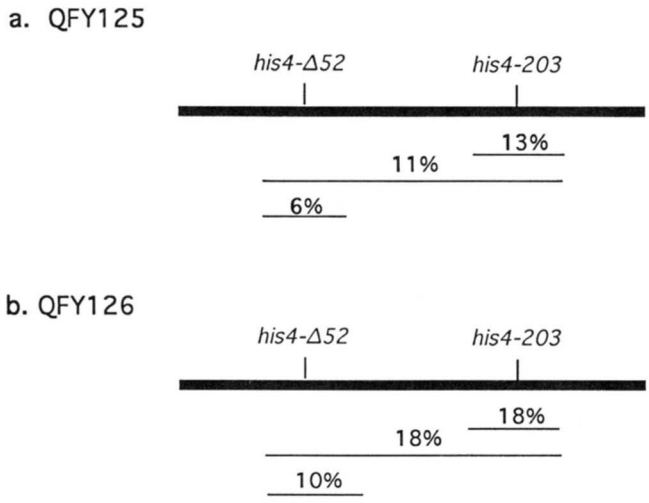


FIGURE 2.—Patterns of conversion and coconversion in QFY125 and QFY126. Lines under each allele indicate the percentage of aberrant segregation for that allele. Lines that include both alleles represent the percentage of coconversion.

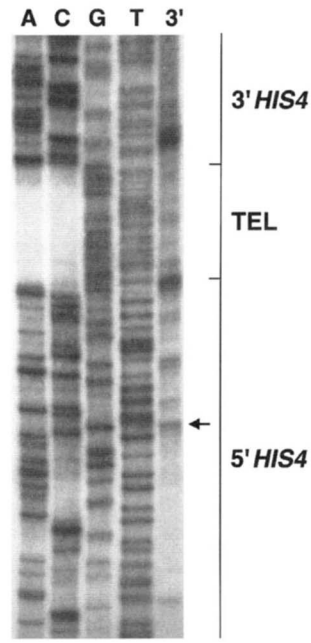


FIGURE 3.—Mapping of double-strand breaks associated with a telomere insertion in the *HIS4* coding region. DNA was isolated from meiotic cells of the strain QFY114. This strain has DSBs associated with the *his4-203* telomere insertion, but does not have the DSB associated with the wild-type hotspot. The high-resolution mapping was performed as described previously (LIU *et al.* 1995; XU and PETES 1996) and as discussed in MATERIALS AND METHODS. The lane marked 3′ represents mapping of the 3′ termini resulting from the hotspot-associated DSBs from one side of the break. The band in this lane indicated by an arrow represented an exogenously added DNA fragment added as a size standard used to help align the DSB sites to the DNA sequence ladder. The lanes labeled A, C, G and T represent the DNA sequence from the region flanking the insertion, prepared as described in MATERIALS AND METHODS.

different tetrad classes in the two strains is significantly different (Fisher exact test, $P < 0.001$).

We constructed *rad50S* derivatives of QFY125 (QFY127) and QFY126 (QFY128) in order to examine the level of DSBs in strains with the *cis* and *trans* arrangement of hotspots. In QFY127, in which the hotspots were located on the same chromosome, only a very weak (<0.5%) DSB was found at the position of the wild-type hotspot and the DSBs at *his4-203* were found at a level of 3.4–4.4% (Figure 1). In QFY128, in which the hotspots were on opposite chromosomes, the level of DSB for the wild-type hotspot was 1–1.9% and the level for the *his4-203* hotspot was 8.1–8.5%. These results confirm the conclusion that the hotspots function more effectively located in *trans* than in *cis*.

Patterns of DSB formation induced by telomeric sequences: Previously, we showed that an insertion of telomeric sequences that replaced the wild-type hotspot resulted in high level of DSBs (FAN *et al.* 1995). Examination of the DSB sites using polyacrylamide gel electrophoresis indicated that the hotspot-associated DSBs occurred within a region of ~50 bp within the *BIK1* sequences adjacent to the telomere insertion (XU and PETES 1996). We find that the *his4-203* telomere insertion results in two DSBs, located near the point of insertion (Figure 1). Fine-structure analysis of these sites shows two strong DSB sites, one near the telomere insertion within the flanking 3′ *HIS4* sequences and one at the boundary of the telomeric and non-telomeric sequences (Figure 3). Similarly, a telomeric insertion upstream of *ARG4* results in two strong DSB sites flanking the insertion (FAN *et al.* 1995).

DISCUSSION

Hotspots inserted in ectopic positions often have activities that are different from their activities at their native position (reviewed by LICHTEN and GOLDMAN 1995). Some of these context effects may represent suppressive interactions between hotspots as described in this and other studies (WU and LICHTEN 1995; XU and KLECKNER 1995). Below, we will discuss: patterns of aberrant segregation and DSB formation observed in strains with heterozygous hotspots in *cis* and *trans*, the relationship of our data to those of others, and possible mechanisms of the hotspot competition.

Patterns of aberrant segregation and DSB formation observed in strains with heterozygous hotspots in *cis* and *trans*: Our analysis of the strains doubly heterozygous for the wild-type and *his4-203* hotspots shows that the level of hotspot activity is greater when the hotspots are located on the opposite chromosomes than when they are on the same chromosome. A calculation of the magnitude of the effects based on the genetic data is complicated. The rate of aberrant segregation measured at each hotspot individually is the sum of three rates: the rate of aberrant segregation initiated at the hotspot, the rate of coconversion events initiated at the adjacent hotspot, and the rate of aberrant segregation

that is independent of hotspot activity. We previously showed that removal of the wild-type hotspot resulted in a level of aberrant segregation of ~15% across the *HIS4* gene, which was termed the basal level of recombination (DETLOFF *et al.* 1992). If this level of aberrant segregation is subtracted from the aberrant segregation rates for both loci in both strains, we calculate that the hotspots are about twofold more active when located in *trans* than when located in *cis*. From the analysis of DSBs, we found ~3.8% (<0.5% for the wild-type hotspot + 3.8% of the *his4-203* hotspot) breaks in QFY125 and 9.7% (1.5 + 8.2%) breaks in QFY126, in reasonable agreement with the genetic data.

The conclusion that there is little hotspot-dependent aberrant segregation at the wild-type hotspot in QFY125 is supported by two other arguments. First, as described in RESULTS, the gene conversion events that involve only *his4-ΔX* show no disparity. Second, only a very weak DSB is observed at the wild-type hotspot in QFY127, the *rad50S* derivative of QFY125 (Figure 1). The hotspot-dependent aberrant segregation events observed in QFY125 probably reflect coconversion events initiated at *his4-203*.

Other studies of interactions between hotspots: Two other detailed studies of interacting hotspots in *S. cerevisiae* have been done. WU and LICHTEN (1995) measured recombination frequencies for mutant *leu2* and *arg4* sequences inserted at various places in the genome, observing considerable context-dependent effects on the rate of recombination. The insertion with *arg4* also had *URA3* and pBR322 sequences. They found DSB sites in the insertion near the *URA3* and pBR322 sequences, but not upstream of *arg4* (the normal location of the hotspot-associated DSB). Deletion of the DSB sites near *URA3* and pBR322 reactivated the DSB site near *ARG4*. In addition, when the *arg4*-containing insertion was placed next to *HIS4*, the level of DSB at the *HIS4* wild-type hotspot was reduced about sevenfold; deletion of the DSB sites within the insertion, restored the DSB at *HIS4*. The *arg4*-containing insertion also had long-range effects. When inserted near *HIS4*, it reduced heteroallelic recombination at *LEU2*, 17 kb away, by about twofold. XU and KLECKNER (1995) examined interactions between two hotspots located ~2 kb apart. They found that the activity of one of these hotspots (site 1), as assayed by measuring DSBs, was elevated 50% by insertion of multiple *Bam*HI linkers at site 1. This insertion reduced the level of DSBs at site 2 about twofold in *cis* and by ~10% in *trans*. The reduction in *trans* was statistically significant at the 90% confidence level.

ROCCO and NICOLAS (1996) examined the effect of nonhomology on the frequency of DSB formation at the *ARG4* hotspot. They showed that nonhomology reduced the frequency of gene conversion and DSB formation, indicating that communication between homologous chromosomes influences the frequency of recombination-initiating events. These results were consistent with the *trans* effects reported by XU and KLECKNER.

Our data are substantially in agreement with these studies, indicating competitive interactions between hotspots at the level of DSB formation. Although our results indicate that this interaction occurs primarily between hotspots located on the same chromosome, we cannot rule out small *trans* effects such as those reported by XU and KLECKNER (1995). Our results indicate that the competition between hotspots as monitored by DSB formation is also reflected by the genetic analysis of hotspot activity (disparity of gene conversion, level of aberrant segregation and crossovers).

Mechanism of suppressive interactions between adjacent hotspots: In our study and those of others (WU and LICHTEN 1995; XU and KLECKNER 1995), the major suppression of hotspot activity occurs when the hotspots are adjacent on the same chromosome. Hotspots in *S. cerevisiae* tend to occur between genes in nuclease-sensitive regions of chromatin (reviewed by LICHTEN and GOLDMAN 1995). These regions often contain binding sites for transcription factors, and these factors are required for hotspot activity at the *HIS4* hotspot (WHITE *et al.* 1993). The observed suppression could reflect a competition between hotspots for proteins required to maintain an open chromatin configuration or the proteins that make the DSBs. It should be noted, however, that WU and LICHTEN (1995) found that the suppressive effects of adjacent hotspots did not necessarily involve a change in the nuclease-sensitivity of chromatin in the hotspot region. The observation that the suppression is much stronger in *cis* than in *trans* could be explained if the factors bind to DNA molecules and diffuse linearly along the DNA until a hotspot is encountered; a similar suggestion was made by WU and LICHTEN (1995). Alternatively, the postulated factors could be located in a local environment near the chromosome in which diffusion is limited. Another possibility is that the interactions may involve loop formation or cooperative alterations of the chromatin structure within one chromosome rather than linear diffusion. Long-range alterations in chromatin structure affecting gene expression have been detected in a variety of systems (WIJGERDE *et al.* 1995).

One important issue is the relationship between chiasma interference and the hotspot interaction described in our studies. Chiasma interference is the suppression of crossovers in one interval by a crossover in an adjacent interval (reviewed by PETES *et al.* 1991; FOSS *et al.* 1993). The degree of hotspot competition is often too great to be accounted for by the effects of a DSB at one site on the probability of formation of DSBs at an adjacent site. For example, WU and LICHTEN (1995) found that an insertion with a frequency of DSBs of 16% suppressed a DSB site in an adjacent sequence (within the *HIS4* gene) about sevenfold. One possibility is that hotspots can exist in two forms, inactive (which

have no chance of receiving a DSB that is processed into a crossover or conversion) and activated (which have a substantial probability of developing a DSB that is processed into a crossover or conversion). By this model, activation of one hotspot interferes with activation of neighboring hotspots, resulting in the observed chiasma interference.

There are several reasons for caution in associating hotspot interference with chiasma interference. First, if hotspot competition is related to chiasma interference, one might expect that all events initiated by DSBs will show interference. Although the issue has not been exhaustively investigated, MORTIMER and FOGEL (1974) presented data indicating that gene conversion events (identified without regard to crossovers) are distributed without interference. Second, chiasma interference can be observed over large physical distances (reviewed by FOSS *et al.* 1993), whereas competitive interactions between hotspots have been examined thus far only for short physical distances (17 kb representing the longest distance examined; WU and LICHTEN 1995). Third, although *zip1* mutants (which lack a component of the synaptonemal complex) have normal levels of gene conversion, they have reduced levels of crossover interference (SYM and ROEDER 1994). The simplest explanation of this result is that interference operates at some stage after DSB formation. One test of the idea that the competitive interactions between adjacent hotspots is related to chiasma interference would be to examine hotspot interactions in a *zip1* mutant strain. Finally, it should be pointed out that no interference was detected for some chromosome intervals in yeast (SYMINGTON and PETES 1988; WHITE and PETES 1994).

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