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Meiosis-Specific Double-Strand DNA Breaks at the *HIS4* Recombination Hot Spot in the Yeast *Saccharomyces cerevisiae*: Control in *cis* and *trans*

QINGQING FAN, FEI XU, AND THOMAS D. PETES*

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

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The region of *Saccharomyces cerevisiae* chromosome III located between the 5′ end of the *HIS4* gene and the 3′ end of the adjacent *BIK1* gene has a very high level of meiotic recombination. In wild-type strains, a meiosis-specific double-strand DNA break occurs in the hot spot region. This break is absent in strains in which the transcription factors Rap1p, Bas1p, and Bas2p cannot bind to the region upstream of *HIS4*. In strains with levels of recombination that are higher than those of the wild type, the break is found at elevated levels. The linear relationship between hot spot activity and the frequency of double-strand DNA breaks suggests that these lesions are responsible for initiating recombination at the *HIS4* recombination hot spot.

A number of meiotic recombination hot spots in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been characterized (30). Hot spots are defined as chromosomal regions associated with unusually high levels of crossovers or aberrant segregation (gene conversion or postmeiotic segregation). Deletion analysis has localized hot spots to small chromosomal regions at the *HIS4* (12, 44, 45), *ARG4* (9, 28, 40), *HIS2* (23), and *THR4* (18) loci in *S. cerevisiae* and at the *ade6* locus of *S. pombe* (31, 42); in addition, hot spots have been observed in yeast chromosomes containing a duplication of *LEU2* (7), an insertion of a Tn*3*-derived transposable element (38), and in a centromere-linked region of chromosome III (41). For many of these hot spots, meiosis-specific double-strand breaks (DSBs) that map near the hot spot have been detected (7, 16, 26, 39, 48, 49). We will discuss in detail only the *ARG4* and *HIS4* hot spots.

At the *HIS4* and *ARG4* loci, the hot spot is located 5′ of the coding sequence (12, 30). This position is consistent with the observed gradient of gene conversion at these loci, with the 5′ end representing the high end of the gradient (12, 15). At the *ARG4* locus, most deletions that remove the region located between positions −139 and +3 cause loss of hot spot activity (9, 28). This region contains a poly(A) tract, and deletion of this tract reduces gene conversion at *ARG4* three- to fourfold (36). There is a meiosis-specific DSB in the region located about 200 bp upstream of the coding sequence (39). In general, deletions that lower the frequency of meiotic recombination also lower the amount of the DSB, although the level of DSB was not precisely correlated with the amount of recombination for all of the deletions (9). In *rad50S* strains, in which the ends resulting from DSBs are not processed (1), the frequency of DSBs at the *ARG4* locus is approximately that expected if these lesions represent the initiating event for meiotic recombination (9, 43).

A deletion of DNA sequences located between the 5′ end of *HIS4* and the 3′ end of the neighboring *BIK1* gene lowers the frequency of meiotic recombination at both loci (12), indicating that this site stimulates recombination bidirectionally. Subsequent studies (32) showed that individual recombination events usually involved either *HIS4* or *BIK1*. Four transcription factors are known to bind upstream of *HIS4*: Gcn4p, Bas1p, Bas2p, and Rap1p (2, 3, 13). In studies of the effects of mutations that eliminate the binding sites for these proteins or the structural genes encoding the proteins, White et al. (45, 46) showed that Bas1p, Bas2p, and Rap1p are required for hot spot activity but Gcn4p is dispensable. The requirement for transcription factors for hot spot activity is not directly related to a requirement for high levels of transcription, because deletion of the TATAA promoter element has no effect on hot spot activity (44). Although the wild-type hot spot requires Bas1p, Bas2p, and Rap1p, duplication of the Rap1p binding site or insertion of yeast telomeric DNA (which binds Rap1p) creates a very strong meiotic recombination hot spot at *HIS4* in the absence of Bas1p and Bas2p (45).

Although in wild-type strains a meiosis-specific DSB at *HIS4* is difficult to see by Southern analysis, in *rad50S* strains, the break near the hot spot region is readily visualized (26). In this report, we examine the frequency of this break in strains with mutations affecting the binding sites of transcription factors or mutations eliminating the transcription factors. We show that the efficiency of hot spot activity measured genetically (aberrant segregation frequency of markers in *HIS4*) correlates well with the level of DSBs in the hot spot region. Mutations that eliminate the Rap1p binding site or the Bas1 or Bas2 protein eliminate the DSB. The recombination events that occur in the absence of hot spot activity (basal level recombination) appear to be independent of a DSB in the hot spot region. In addition, we show that one of the haploid parents of our diploid strain can be induced to form a DSB at the same position as in the diploid because the haploid has an *rme1* mutation.

MATERIALS AND METHODS

Plasmids. All of the plasmids used in this study, except for those specifically noted, contained yeast DNA insertions in the *Bam*HI sites of cloning vectors YIp5 and B142 (a derivative of YIp5 that lacks the *Pvu*II site). The names of the plasmids, the mutant alterations contained in the plasmids, and the references for their construction are summarized as follows: pC1G17 (*his4-Sal* [46]), p42 (*his4-ATC* [10]), pDN13 (*his4-lopc* [45]), pDN4 (*his4-lop* [46]), pDN22 (*his4-IR9* [45]), pPD5 (*his4-Δ52* [45]), pPD8 (*his4-flp* [46]), pMW35 (*his4-51* [46]), pMW50 (*his4-202*; telomere insertion upstream of *HIS4* [45]), pMW53 (*arg4-tel* [45]), pMW55 (*his4-204*; oligonucleotide with two Rap1p binding sites inserted upstream of *HIS4* [45]), pMW56 (*his4-205*; oligonucleotide with one wild-type and one mutant Rap1p binding site inserted upstream of *HIS4* [45]), AB289 (*bas1-1*

* Corresponding author. Phone: (919) 962-1445. Fax: (919) 962-1625.

TABLE 1. Haploid derivatives of strain AS4

Name	Upstream alteration	Alteration of coding sequence	Other change(s)	Parental strain	Plasmid used to insert alteration	Name of *rad50S* derivative
AS4	WT[a]	WT	WT			DNY107
PD63	*his4-Δ52*	WT	WT	AS4	pPD5	
MW30	*his4-51*	WT	WT	AS4	pMW35	HF2
PD104	*his4-flp*	WT	WT	AS4	pPD8	QFY17
MW78	WT	WT	*bas1-1*	AS4	AB289	QFY4
MW71	WT	WT	*bas2-2*	AS4	AB328	QFY6
MW73	*his4-202*	WT	WT	PD63	pMW50	HF6
MW82	*his4-204*	WT	WT	PD63	pMW55	QFY9
MW83	*his4-205*	WT	WT	PD63	pMW56	QFY11
QFY13	WT	WT	*bas1-1 bas2-2*	MW71	AB289	QFY15

[a] WT, wild type.

[45]), and AB328 (*bas2-2* [45]). Plasmids AB289 and AB328 were provided by K. Arndt (Cold Spring Harbor Laboratory), and p42 was provided by T. Donahue (Indiana University).

The plasmid pDN42 (used for Southern analysis) had 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(−) (26). The plasmid pHY10-1 (provided by A. Mitchell, Columbia University) had an *ime1* deletion mutation cloned in a vector with a *URA3* marker. The plasmid pLS88 had a 4-kb *Hin*dIII fragment (containing the region of chromosome III with the wild-type allele of the gene affecting sporulation in AS4) cloned into YCp50 (41). The plasmid pSG205 (provided by C. Atcheson, University of Chicago) had a *Hin*dIII fragment with the *RAD50* gene cloned in YCp50. The plasmid 3C3-1 (also called pAM204, obtained from A. Mitchell, Columbia University) had the *RME1* gene cloned into YCp50 (24). The plasmid pDN47 (provided by D. Nag, New York State Department of Health) was derived from the plasmid pCA9 (provided by C. Atcheson, University of Chicago) by substituting the *URA3* insertion disrupting *SPO11* with a *LEU2* insertion. The plasmid pAK1 (provided by A. Kazantsev, University of North Carolina) contained a 3-kb *Pst*I-*Hin*dIII fragment with the *ARG4* gene and flanking sequences in the vector YRplac33.

Yeast strains. Most of the yeast strains in this study were derived by transformation from the haploid strains AS4 (**a** *trp1 arg4-17 tyr7 ade6 ura3*) and AS13 (α *leu2 ade6 ura3*) described previously (38). The construction of various haploids is summarized in Tables 1 and 2. All alterations were done with the two-step transplacement procedure (35), except for the insertion of the *rad50S* mutation, which was done by one-step transplacement (34). To construct *rad50S* derivatives of various haploid strains, we treated pNKY349 with *Eco*RI and *Bam*HI, and selected Ura$^+$ transformants. The resulting transformants were screened for sensitivity to methyl-methane sulfonate (1).

Diploid strains, *RAD50*, and isogenic *rad50S* derivatives, were constructed by the following crosses (*RAD50* strain listed first): (i) DNY48 (AS4 × DNY47), QFY105 (DNY107 × QFY7); (ii) DNY26 (AS4 × DNY25), FX3 (DNY107 × HF4); (iii) DNY11 (AS4 × DNY9), FX1 (DNY107 × HF1); (iv) MW118 (MW30 × MW33), FX2 (HF2 × HF3); (v) PD106 (PD104 × PD105), QFY110 (QFY17 × QFY16); (vi) MW154 (MW73 × MW72), FX4 (HF6 × HF5); (vii) MW160 (MW81 × MW79), FX6 (HF8 × HF7); (viii) QFY101 (MW82 × QFY1), QFY106 (QFY9 × QFY8); (ix) QFY102 (MW83 × QFY2), QFY107 (QFY11 × QFY10); (x) MW158 (MW78 × MW77), QFY103 (QFY4 × QFY3); (xi) MW153 (MW71 × MW67), QFY104 (QFY6 × QFY5); (xii) QFY108 (QFY13 × QFY12), QFY109 (QFY15 × QFY14); and (xiii) PD84 (AS4 × PD74).

Several strains were used for analysis of meiosis-specific DSBs at *ARG4*. The haploid strain MW81 was derived from AS4 by two-step transplacement with the plasmid pMW53, resulting in the insertion of telomeric sequences upstream of *ARG4* (45); similarly, the haploid strain MW79 was derived from AS13. The diploid strain MW160 was made by crossing MW81 and MW79. *rad50S* derivatives of the haploid strains (HF8 derived from MW81 and HF7 derived from MW79) were constructed with the plasmid pNKY349, as described above; the diploid FX6 was made by crossing HF7 and HF8. The wild-type level of *ARG4* DSBs was examined in the diploid DNY115, constructed by crossing *rad50S* haploid derivatives of AS4 (DNY107) and AS13 (DNY106).

The remaining yeast strains were constructed for analysis of haploid-specific DSBs. The *rad50S* haploid strains HF5 and HF6 had telomeric insertions upstream of *HIS4*. Derivatives of these strains with *ura3* mutations (HF5U and HF6U) were isolated by plating the strains on medium containing 5-fluoroorotate (5); the diploid constructed by mating HF5U and HF6U was FX4U. The mutation in HF6U that affects sporulation (41) was complemented by transforming HF6U with the plasmid pLS88, resulting in the strain HF13. An *ime1* mutation was introduced into HF5U (resulting in the strain HF9) by using the plasmid pHY10-1 by two-step transplacement. An *spo11* derivative of HF5U (HF11) was generated by one-step transplacement with an *Sph*I-*Hin*dIII digest of pDN47 DNA. A sporulation-competent (*RAD50*) derivative of FX4U (FX7) was constructed by transforming FX4U with the plasmid pSG205. An *RME1* derivative of HF5U (HF15) was constructed by transformation with the plasmid 3C3-1. To examine the complementation of the mutation in HF5 with *rme1*, we

TABLE 2. Haploid derivatives of strain AS13

Name	Upstream alteration	Alteration of coding sequence	Other change(s)	Parental strain	Plasmid used to insert alteration	Name of *rad50S* derivative
AS13	WT[a]	WT	WT			
DNY47	WT	*his4-IR9*	WT	AS13	pDN22	QFY7
MW1	WT	*his4-Sal*	WT	AS13	pC1G17	
PD74	WT	*his4-ATC*	WT	AS13	p42	
DNY25	WT	*his4-lopc*	WT	AS13	pDN13	HF4
DNY9	WT	*his4-lop*	WT	AS13	pDN4	HF1
PD57	*his4-Δ52*	WT	WT	AS13	pPD5	
MW62	WT	WT	*bas2-2*	AS13	AB328	
MW77	WT	*his4-IR9*	*bas1-1*	DNY47	AB289	QFY3
PD105	*his4-flp*	*his4-Sal*	WT	MW1	pPD8	QFY16
MW33	*his4-51*	*his4-lop*	WT	DNY9	pMW35	HF3
MW68	*his4-202*	WT	WT	PD57	pMW50	
PD80	*his4-Δ52*	*his4-lopc*	WT	PD57	pDN13	
MW67	WT	*his4-IR9*	*bas2-2*	MW62	pDN22	QFY5
MW72	*his4-202*	*his4-lopc*	WT	MW68	pDN13	HF5
QFY1	*his4-204*	*his4-lopc*	WT	PD80	pMW55	QFY8
QFY2	*his4-205*	*his4-lopc*	WT	PD80	pMW56	QFY10
QFY12	WT	*his4-IR9*	*bas1-1 bas2-2*	MW67	AB289	QFY14

[a] WT, wild type.

fused HF5 with the tester strain DH223 (**a** *trp1 his3 ade2-10 rme1*::*LEU2 FUS1-lacz* fusion; obtained from K. Tatchell, North Carolina State University) to create the diploid FX23.

Media and genetic techniques. In general, standard procedures for mating, transformation, and dissection were used (37). As noted in the Results section, cells were sporulated either at 18°C on solid sporulation medium (10) or at 25°C in liquid medium under the conditions specified previously (26). Strains were usually dissected after 2 to 3 days in sporulation medium at 25°C or after 3 to 5 days in sporulation medium at 18°C. Aberrant segregation of heterozygous *HIS4* mutations was monitored by examining the colonies microscopically in order to detect small sectors. To fuse cells of the same mating type, we mixed spheroplasts derived from the two strains under the conditions described for transformation by Becker and Guarente (4).

Analysis of DSBs in DNA. DNA was isolated from cells sporulated in 1% potassium acetate at 25°C by methods described by Goyon and Lichten (19) and Nag and Petes (26). In brief, for each time point, about 3×10^8 cells were harvested by centrifugation and treated with Zymolyase. The resulting spheroplasts were lysed with detergent and treated with proteinase K and RNase. The DNA was precipitated and washed with ethanol. To examine the DSBs at *HIS4* or *ARG4*, we treated the DNA with *Bgl*II and the resulting fragments were separated on a 0.8% agarose gel. Standard Southern analysis was performed with the intact plasmid pDN42 (*HIS4*) or a 1-kb *Eco*RV-*Bgl*II fragment derived from pAK1 (*ARG4*) as the hybridization probe. Quantitation of the resulting blots was done with a Molecular Dynamics phosphorimager with procedures recommended by the manufacturer.

Statistical methods. Comparisons between classes of events were done with a contingency chi-square test or a Fisher exact test (when the classes had fewer than five events). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Hot spot activity in strains with sequence alterations upstream of *HIS4*. The *HIS4-BIK1* region is shown in Fig. 1. In previous studies (45, 46), we showed that strains with mutations in the Rap1p binding site upstream of *HIS4* or that lacked Bas1p or Bas2p showed levels of aberrant segregation of 15 to 18% for palindromic markers inserted near the beginning of the *HIS4* gene. Although this recombination rate is high relative to those in some areas of the yeast genome, it is about two- to threefold reduced from the level of aberrant segregation in the wild-type strain; in addition, because these mutational changes eliminate the polarity gradient at *HIS4* (12), these alterations eliminate hot spot activity. We also found that an insertion of telomeric sequences replacing the normal upstream region of *HIS4* resulted in a very strong recombination hot spot (45). Because telomeric sequences bind Rap1p (6) and iterated nontelomeric Rap1p binding sites stimulate recombination when inserted heterozygously in front of *HIS4*, we suggested that duplicated Rap1p binding sites could function as a strong recombination hot spot, although a single Rap1 binding site was insufficient.

Several of the studies cited above, in which we examined the effects of various oligonucleotides containing Rap1p binding sites, were done with heterozygous insertions. Because heterozygosity of the insertion might influence the ability to initiate a recombination event, we have examined the effects of homozygous insertions that have one or more Rap1p binding sites. In one strain, QFY101, an oligonucleotide containing a duplication of the Rap1p binding site in front of *HIS4* was used to replace the normal upstream sequences on both homologs. The frequency of aberrant segregation (63%) of the heterozygous marker in *HIS4* (*his4-lopc*) in this strain was extraordinarily high (Table 3). This level of recombination was close to that observed in a strain (MW154) with the telomeric insertion in front of *HIS4* (72%). The strain QFY102 had the same insertion as QFY101, except that one of the Rap1p binding sites was disrupted by four single-base-pair changes. In this strain, the aberrant segregation frequency of *his4-lopc* was reduced to 29%. Although the frequency of aberrant segregation was clearly lowered by eliminating one of the Rap1p binding sites, it was higher than the 15 to 18% observed in

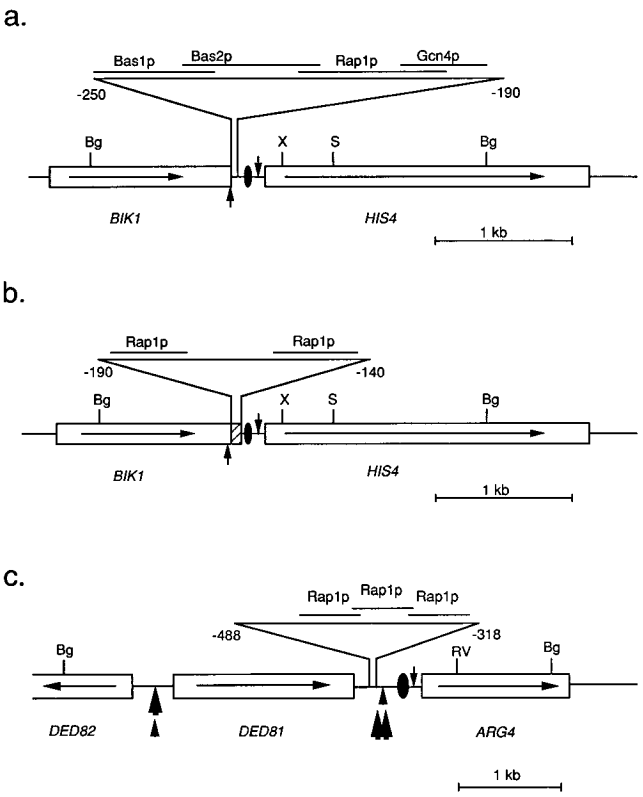

FIG. 1. Physical maps of the *HIS4* and *ARG4* recombination hot spots (based partly on data derived from references 2, 3, 9, 13, and 39). Coding sequences are indicated by rectangles, and the directions of transcription are indicated by arrows within the rectangles. Solid ovals represent TATAA sequences. Vertical arrows above the map indicate the start of transcription, whereas vertical arrows below the map indicate the positions of DSBs. Expanded regions above the linear map represent protein-binding sites with the approximate positions of the binding proteins indicated by horizontal lines. The numbers associated with the expanded regions represent the distance to the initiation codon of *HIS4* or *ARG4*. Restriction enzymes: X, *Xho*I; Bg, *Bgl*II; S, *Sal*I; RV, *Eco*RV. (a) Map of the wild-type hot spot region associated with *HIS4*. The hybridization probe used to detect the DSB (pDN42) contained sequences between the *HIS4 Xho*I and *Bgl*II sites. Mutations used to monitor aberrant segregation were located at the *Sal*I site. (b) Map of the hot spot associated with two Rap1p binding sites upstream of *HIS4*. In these strains, the wild-type binding sites upstream of *HIS4* were deleted and replaced with an oligonucleotide containing two Rap1p binding sites (45). Such strains have elevated hot spot activity. (c) Map of the *ARG4* hot spot with and without a telomeric insertion. The positions of DSBs in the wild-type strain are indicated by the vertical arrows, located below the map, closest to the horizontal line; the positions of DSBs in the strain with the telomeric insertion are shown by the vertical arrows located below the arrows representing the wild-type DSBs. The sizes of the arrows reflect the frequency of the DSBs.

strains with disruptions of Bas1p and Bas2p (MW158 and MW153 [Table 3]). Thus, although a single Rap1p binding site in the context of the wild-type hot spot does not stimulate recombination in the absence of Bas1p or Bas2p, the single effective Rap1p binding site in QFY102 appears to have some hot spot activity in the absence of the other protein binding sites.

One explanation of these observations is that the binding of Rap1p is necessary for wild-type hot spot activity; although the binding of both Bas1p and Bas2p facilitates the binding of Rap1p, the binding of Bas1p in the absence of Bas2p or vice versa prevents the binding of Rap1p. This model makes the prediction that strains that have had both Bas1p and Bas2p deleted will have normal hot spot activity. We found, however, that the *bas1 bas2* double-mutant strain (QFY108) had about

TABLE 3. Aberrant segregation patterns of *HIS4* markers at 18 and 25°C

Temp	Strain (*rad50S* derivative)	Relevant genotype	No. of tetrads in each class						Total no. of tetrads	% Aberrant segregation	% DSBs[b]
			6:2	2:6	5:3	3:5	Aberrant 4:4	Other aberrant segregation[a]			
18°C	MW118[c] (FX2)	*his4-51 his4-lop* *his4-51 HIS4*	7	2	20	24	2	4	325	18	ND
	MW158[d] (QFY103)	*bas1-1 his4-IR9* *bas1-1 HIS4*	7	6	16	15	5	1	335	15	ND
	MW153[d] (QFY104)	*bas2-2 his4-IR9* *bas2-2 HIS4*	3	5	14	14	4	2	247	17	ND
	QFY108 (QFY109)	*bas1-1 bas2-2 his4-IR9* *bas1-1 bas2-2 HIS4*	1	8	22	12	3	1	288	16	ND
	QFY102 (QFY107)	*his4-205 his4-lopc* *his4-205 HIS4*	14	10	33	28	8	1	329	29	ND
	DNY11[e] (FX1)	*his4-lop* *HIS4*	5	6	18	13	4	6	117	44	ND
	DNY26[e] (FX3)	*his4-lopc* *HIS4*	30	16	89	72	15	24	494	50	ND
	DNY48[e] (QFY105)	*his4-IR9* *HIS4*	14	8	28	26	3	6	185	46	ND
	PD84[f]	*his4-ATC* *HIS4*	113	33	56	57	3	40	677	45	ND
	QFY101 (QFY106)	*his4-204 his4-lopc* *his4-204 HIS4*	16	19	50	45	23	17	268	63	ND
	MW154[d] (FX4)	*his4-202 his4-lopc* *his4-202 HIS4*	17	9	56	72	20	57	321	72	ND
25°C	MW118 (FX2)	*his4-51 his4-lop* *his4-51 HIS4*	1	1	2	6	0	1	100	11	<0.2
	MW158 (QFY103)	*bas1-1 his4-IR9* *bas1-1 HIS4*	0	3	8	3	1	0	113	13	<0.2
	MW153 (QFY104)	*bas2-2 his4-IR9* *bas2-2 HIS4*	1	1	3	7	1	0	117	11	<0.2
	QFY108 (QFY109)	*bas1-1 bas2-2 his4-IR9* *bas1-1 bas2-2 HIS4*	3	2	2	6	1	1	113	13	<0.2
	QFY102 (QFY107)	*his4-205 his4-lopc* *his4-205 HIS4*	0	2	6	7	1	1	103	17[g]	1.7
	DNY11 (FX1)	*his4-lop* *HIS4*	5	1	10	9	1	0	111	23[g]	5
	DNY26 (FX3)	*his4-lopc* *HIS4*	7	7	13	20	2	1	257	19[g]	2.6
	DNY48 (QFY105)	*his4-IR9* *HIS4*	4	2	10	10	0	1	120	23[g]	4.4
	PD84	*his4-ATC* *HIS4*	10	4	3	10	0	1	92	30[g]	ND
	QFY101 (QFY106)	*his4-204 his4-lopc* *his4-204 HIS4*	7	6	11	20	7	4	104	53	16
	MW154 (FX4)	*his4-202 his4-lopc* *his4-202 HIS4*	9	2	17	15	4	14	98	62	19

[a] Includes 8:0, 0:8, 7:1, 1:7, aberrant 6:2, and aberrant 2:6, as well as a small number of tetrads with three postmeiotic segregation events.
[b] Percentage of label in DSB-specific band relative to total as determined by Southern analysis of *Bgl*II-treated DNA isolated from cells incubated for 24 h in sporulation medium. DNA was isolated from isogenic *rad50S* derivatives in all cases (for example, the percentage of DSBs for MW118 was determined with the strain FX2). Each value is based on two or more independent experiments. ND, not determined.
[c] Reference 46.
[d] Reference 45.
[e] Data on these strains were reported previously (25, 27). In the previous publications, however, sectors were not examined microscopically. Consequently, these dissections were repeated.
[f] Reference 11.
[g] Statistically significant ($P<0.05$) decrease in aberrant segregation frequency at 25°C compared with 18°C (determined by chi-square contingency test).

the same level of aberrant segregation (16%) as that observed for strains with the single mutations (Table 3).

The results described above indicate that, in some chromosomal contexts, a single Rap1p binding site can contribute to hot spot activity, whereas in other contexts, a single Rap1p binding site has no effect. These context effects may be related to the binding of proteins at nearby sites. For example, in strains with the wild-type upstream region, the Rap1p binding site overlaps with a high-affinity Gcn4p binding site (13); this Gcn4p binding site is not present in QFY102. Thus, the difference in recombination activity observed with QFY102 compared with that of MW153 and MW158 may reflect an interaction between Rap1p and Gcn4p (or some other DNA-binding protein).

Recombination levels at 18 and 25°C. We previously found that meiotic recombination rates in our genetic background were very high when the cells were sporulated at 18°C. The efficiency of sporulation on plates at 18°C (about 10%), how-

TABLE 4. Comparisons of crossovers in strains sporulated at 18 and 25°C

Strain	Relevant genotype	Hot spot activity[a]	Plate sporulation at 18°C				Liquid sporulation at 25°C			
			No. of tetrads in each class[b]			Map distance (cM)[c]	No. of tetrads in each class[b]			Map distance (cM)[c]
			PD	NPD	T		PD	NPD	T	
MW118[d]	*his4-51 his4-lop* *his4-51 HIS4*	−	188	1	112	20	57	1	31	21
MW158[e]	*bas1-1 his4-IR9* *bas1-1 HIS4*	−	185	4	110	22	81	0	27	13[f]
MW153[e]	*bas2-2 his4-IR9* *bas2-2 HIS4*	−	144	4	70	22	69	2	37	23
QFY108	*bas1-1 bas2-2 his4-IR9* *bas1-1 bas2-2 HIS4*	−	184	3	69	17	74	0	26	13
QFY102	*his4-205 his4-lopc* *his4-205 HIS4*	±	176	3	107	22	63	0	32	17
DNY11	*his4-lop* *HIS4*	+	42	3	54	36	67	1	33	19[f]
DNY26	*his4-lopc* *HIS4*	+	189	6	214	31	180	9	116	28[f]
DNY48	*his4-IR9* *HIS4*	+	86	3	65	27	55	3	51	32
PD84[g]	*his4-ATC* *HIS4*	+	116	3	102	27	49	2	22	23[f]
QFY101	*his4-204 his4-lopc* *his4-204 HIS4*	++	75	11	127	45	34	4	48	42
MW154[e]	*his4-202 his4-lopc* *his4-202 HIS4*	++	75	23	133	59	30	3	45	40

[a] −, no hot spot activity; ±, weak hot spot activity; +, wild-type hot spot activity; ++, elevated hot spot activity.
[b] PD, parental ditype; NPD, nonparental ditype; T, tetratype.
[c] Calculated by using the Perkins equation (29). cM, centimorgans.
[d] Reference 46.
[e] Reference 45.
[f] Significant ($P < 0.05$) difference in number of tetrads in each class at 18 and 25°C as determined by Fisher's exact test.
[g] Reference 11.

ever, was considerably poorer than the efficiency of sporulation in liquid medium at 25°C (about 40% [26]). Thus, our physical analysis of DSBs was done with cells sporulated at 25°C in liquid medium. In order to compare levels of DSBs with the frequency of meiotic recombination assayed genetically, we did tetrad dissection of strains sporulated at 25°C in liquid. The results of this analysis are shown in Table 3.

Several generalizations about these data can be made. First, similar patterns of aberrant segregation are observed at the two temperatures. For example, strains with wild-type upstream sequences have higher levels of aberrant segregation than strains with mutations in the Rap1p binding site or strains with *bas1* and/or *bas2* mutations. In addition, strains with telomeric insertions upstream of *HIS4* have the highest level of aberrant segregation at both temperatures. Second, the levels of aberrant segregation at *HIS4* are higher at 18°C than at 25°C for all strains, although this elevation is not statistically significant for all strains. For all strains with wild-type upstream sequences (DNY11, DNY26, DNY48, and PD84), the level of aberrant segregation is significantly elevated at 18°C relative to 25°C. In none of the other strains, except QFY102, is the difference in aberrant segregation frequencies at the two temperatures significant.

We also monitored the frequency of crossovers in the *HIS4-LEU2* interval as a function of the temperature of sporulation (Table 4). The frequency of crossovers was significantly higher at 18°C for strains DNY11, DNY26, MW158, and PD84. As described above, three of these strains (DNY11, DNY26, and PD84) also had significantly elevated levels of aberrant segregation. Because aberrant segregation events (postmeiotic segregation and gene conversion) are often associated with crossovers of flanking markers (30), this type of correlation is expected. However, there were also strains in which this correlation was not observed. In strains DNY48 and QFY102, the frequencies of aberrant segregation but not the frequencies of crossing over were significantly elevated at 18°C. In strain MW158, the frequency of crossovers but not the frequency of aberrant segregation was significantly elevated at 18°C.

There are three explanations for the differential effects of temperature on the frequency of aberrant segregation and crossing over in some of the strains. First, it is possible that aberrant segregation and crossing over involve different intermediates that are affected differently by temperature in some strains. Alternatively, the direction of resolution of a joint molecule could be affected differently by temperature in the different strains. Third, it should be pointed out that the statistical analyses for aberrant segregation and crossovers were done differently. For the analysis of aberrant segregants, we compared (by 2-by-2 contingency chi-square analysis) the number of tetrads with aberrant segregation patterns with the total number of tetrads at the two temperatures; for the analysis of crossovers, we compared the numbers of tetrads with various segregation patterns (tetratype and nonparental ditype asci) with the total number of tetrads. Thus, finding a significant difference for one type of analysis does not ensure that a significant difference will be observed with a different type of analysis in the same strain.

Double-strand DNA breaks at *HIS4* and *ARG4* in diploid strains. We found previously (26) that a meiosis-specific DSB was present at *HIS4*. In *RAD50* strains, this band of hybridiza-

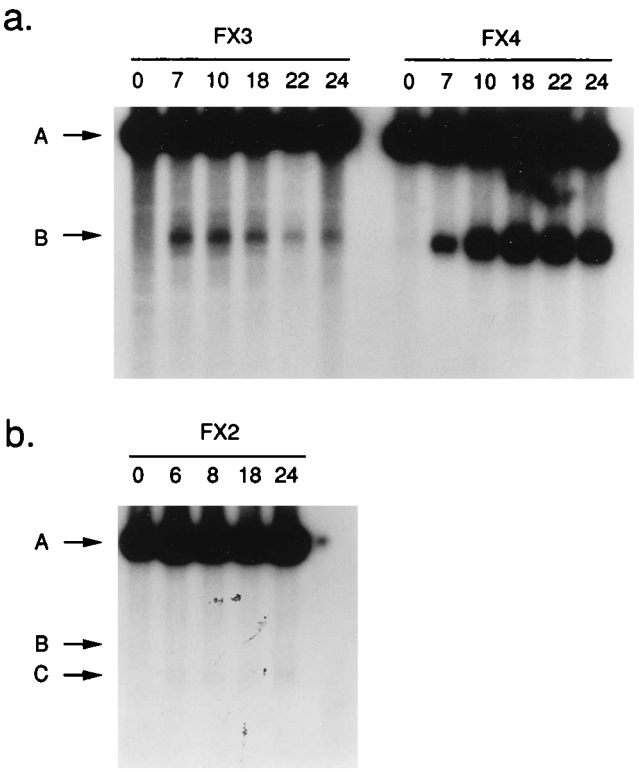

FIG. 2. Southern analysis of meiosis-specific DSBs in strains with different levels of recombination hot spot activity. DNA was isolated from cells incubated for various times in sporulation medium (time in hours is shown above each lane) and treated with *Bgl*II. The resulting fragments were examined by Southern analysis with pDN42 as a hybridization probe. The arrows marked A represent the positions of the *Bgl*II fragment without a meiotic DSB, and those marked B represent the positions (Fig. 2a) or expected positions (Fig. 2b) of the DSB at the hot spot. (a) DSBs in a strain with the wild-type hot spot (FX3) and a strain with elevated hot spot activity (FX4). The FX4 strain has elevated activity as a consequence of a telomeric insertion upstream of *HIS4*. (b) Southern analysis of FX2, a strain with a mutation in the Rap1p binding site upstream of *HIS4*. No DSB is detected at the normal position (arrow B), but a very small amount of DSB is observed at a new position (arrow C).

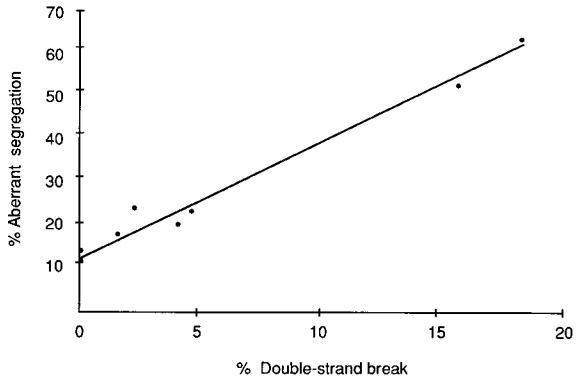

FIG. 3. Relationship between the level of aberrant segregation and the level of meiosis-specific DSBs at the *HIS4* locus. This figure is based on information derived from Table 3 (aberrant segregation frequency at 25°C and percentage of DSBs in *rad50S* derivatives); all of the strains in Table 3 were used for the datum points except PD84.

tion representing the DSB was fuzzy and transient. In a strain with a *rad50S* mutation, the break was much more discrete, as expected from previous studies (1). The amount of this break in a strain with wild-type sequences upstream of *HIS4* was about 3 to 5% of the total DNA (Fig. 2a and Table 3). The size of the DNA fragment representing the DSB in a *Bgl*II digest is about 2 kb, representing a cleavage near the end of *BIK1* (Fig. 1a). In most strains, DSBs were observed about 4 to 6 h after incubation of the cells in sporulation medium. Because the amount of DSB as a fraction of total DNA reaches a plateau at about 18 h, we compared the levels of DSBs in different strains by using DNA isolated from cells incubated for 24 h in sporulation medium.

Strains that lacked a Rap1p binding site (MW118), Bas1p (MW158), Bas2p (MW153), or both Bas1p and Bas2p (QFY108) had relatively low (11 to 13% when sporulated at 25°C) levels of aberrant segregation for *HIS4* markers (Table 3), indicating that these strains lacked hot spot activity. We found that *rad50S* derivatives of these strains also lacked a DSB at the normal site (Fig. 2b). We estimated that we could detect approximately 1/10th to 1/20th the normal DSB level. In one strain (FX2) that lacked the normal DSB, there was a faint band of about 1.65 kb that presumably represented a DSB within the *HIS4* gene (Fig. 2b). This break was not evident in either wild-type strains or two other strains (QFY103 and QFY104) that lacked hot spot activity.

As discussed above, strain QFY102, in which the normal upstream sequences of *HIS4* were replaced with an oligonucleotide with one mutant and one wild-type Rap1p binding site, had a level of hot spot activity (17% aberrant segregation at 25°C) intermediate between that observed for wild-type strains and that observed for the strains with no hot spot activity. The *rad50S* derivative of this strain (QFY107) also had a low amount (1.7%) of DSB compared with that observed in the wild-type control strains (3 to 5% in strains FX3, QFY105, and FX1).

Two strains, MW154 and QFY101, had levels of aberrant segregation at *HIS4* that were considerably higher than those observed in wild-type strains. In MW154, a region of about 170 bp of the normal upstream region was deleted and replaced with 69 bp of telomeric sequence (45). In QFY101, the same deletion was replaced with a 51-bp oligonucleotide containing two Rap1p binding sites (45). The high levels of aberrant segregation observed for *HIS4* markers in these strains (62% for MW154 and 53% for QFY101) were correlated with high levels of DSBs (Fig. 2a) in the *rad50S* derivatives (19% for FX4 and 16% for QFY106). The position of the DSB in MW154 and QFY101 is approximately at the same location as that seen in wild-type strains. This location is near the Rap1p binding site that is most distal to *HIS4*. The data related to the levels of aberrant segregation and DSB at *HIS4* are summarized in Table 3 and Fig. 3. There is a linear relationship between the level of DSB and the frequency of aberrant segregation.

We also examined DSBs at *ARG4* for two reasons. First, in strains in which the *HIS4* DSB was not present, the existence of a DSB at *ARG4* was an important demonstration that the strain was capable of generating DSBs; this control was used with all strains. Second, we wanted to determine whether the level of DSB at *ARG4* was proportional to the frequency of aberrant segregation at this locus. In *rad50S* strains, with wild-type sequences upstream of *ARG4*, two DSBs were observed (Fig. 4). One break was located about 150 to 200 bp upstream of the *ARG4* initiating codon; this break is presumably that reported in previous studies mapping at −185 to −200 (9, 39, 40). A second break was observed approximately 2 kb away from the first. This break corresponds in position to the DSB mapped upstream of the *DED81* gene (9, 39). In our strain background, we found approximately the same frequency of

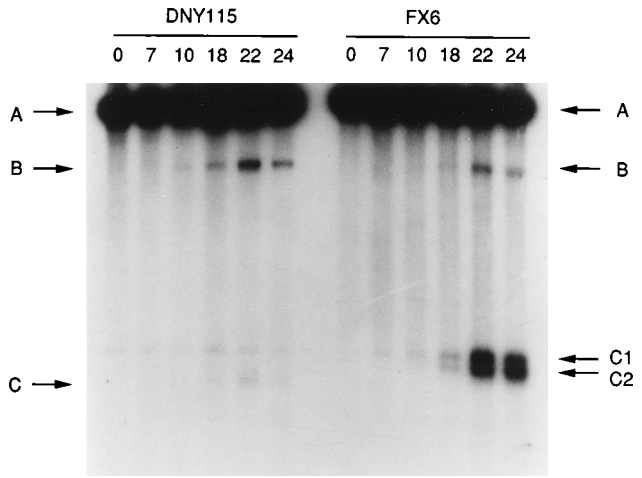

FIG. 4. DSBs at the *ARG4* locus in a wild-type strain and in a strain with an elevated level of hot spot activity. DNA was isolated from cells incubated in sporulation medium for various times (time in hours is shown above each lane) and treated with *Bgl*II. The resulting fragments were separated, transferred to a membrane, and hybridized to a probe derived from pAK1. Arrows A and B represent the unbroken *Bgl*II fragment and the DSB near the *DED81* gene (Fig. 1c), respectively. Strain DNY115 has the wild-type *ARG4* hot spot. Arrow C indicates the position of the DSB associated with *ARG4* in this strain. Strain FX6 has a telomeric insertion upstream of *ARG4* that stimulates recombination. This hot spot is associated with high levels of two DSBs, indicated by arrows C1 and C2.

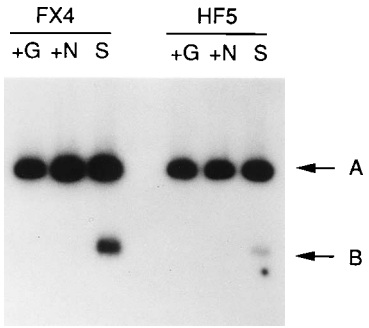

FIG. 5. DSBs associated with a wild-type diploid (FX4) and one of its haploid parental strains (HF5). These two strains were incubated in sporulation medium (S), sporulation medium plus glucose (G), or sporulation medium plus a nitrogen source (N) for 24 h. DNA was isolated from each sample and analyzed for the level of DSB as described in the legend to Fig. 2. Arrows A and B represent the position of the unbroken *Bgl*II fragment and the position of the DSB, respectively.

gene conversion at *arg4-17* (8%) as that observed in previous studies (15). In addition, the percentage of chromosomes with a DSB at *ARG4* in our wild-type background (2%) was similar to that observed by others (2% ± 0.6% [9]). The DSB at *DED81* was stronger than that observed at *ARG4*.

Insertion of telomeric sequences (the same insertion used at *HIS4*) at a position 319 bp upstream of the initiating codon in *ARG4* dramatically elevated the frequency of gene conversion of *arg4-17* (45). In these studies, the strain homozygous for the *arg4-tel* allele (MW160) was sporulated at 18°C and had a conversion frequency of 52% for the heterozygous *arg4-17* mutation. We repeated the meiotic analysis for MW160 cells sporulated in liquid at 25°C and found a frequency of conversion of 33%. The level of DSB in the *rad50S* derivative of MW160 (FX6) was elevated approximately proportionally to the level of gene conversion; the average level of DSB was 6% compared with the 2% level observed in wild-type strains. Interestingly, there were two closely spaced breaks induced by the telomeric insertion near *ARG4*, and the break at the normal position was reduced or eliminated (Fig. 1c and 4).

Double-strand DNA breaks in haploid strains. The experiments described above were done with diploid strains made by crossing derivatives of the haploid strain AS4 with derivatives of the haploid strain AS13. One such diploid strain was FX4, which is homozygous for the telomeric insertion upstream of *HIS4*, heterozygous for a palindromic insertion in the *HIS4* coding sequence (*his4-lopc*), and homozygous for *rad50S*. This strain has a very high level of meiosis-specific DSB (Table 3). As a control, we incubated the haploid parents of FX4 (strains HF5 and HF6) in sporulation medium. DNA isolated from the haploid strain HF5 (derived from strain AS13) contained a DSB at approximately the same position as that observed in the diploid FX4 (Fig. 5), whereas DNA isolated from strain HF6 (derived from strain AS4) did not contain breaks (data not shown). Strains derived from AS4 (including HF6) contain a mutation in a centromere-linked gene on chromosome III that prevents sporulation in homozygous diploids (41). We constructed a derivative of HF6 (HF13) in which this mutant defect was complemented by a plasmid. In this strain, no DSB was observed in DNA isolated after incubation of the cells in sporulation medium. Therefore, the appearance of a DSB in cells incubated in sporulation medium is a property of the AS13 but not the AS4 genetic background.

We next investigated whether the DSB observed in HF5 had the properties expected for a meiosis-specific DSB. Meiosis is repressed by glucose or a nitrogen source in the sporulation medium (14). We found that addition of either glucose or ammonium sulfate prevented formation of the DSB (Fig. 5). Sporulation is blocked by a number of mutations, including *ime1* and *spo11*. When either of these mutations was introduced into HF5 (generating strains HF9 [*ime1* derivative of HF5] and HF11 [*spo11* derivative of HF5]), no DSBs were observed (data not shown). Thus, the double-strand DNA breaks in HF5 appear to be induced by a pathway that mimics the normal meiotic induction.

Meiosis and sporulation are usually properties of diploid strains that express both **a** and α mating-type information. Mutations in *rme1*, however, allow **a**/**a** or α/α strains to sporulate and allow the early steps of meiosis to occur in haploid strains (20, 21). The *rme1* mutation has been found as a naturally occurring variant in several laboratory strains (20). To investigate whether HF5 contained an *rme1* mutation, we constructed a diploid by fusing HF5 (**a** mating type) to a tester strain DH223 (**a** mating type with an *rme1* mutation). The diploid strain (FX23) mated as an **a** strain and sporulated. All spores derived from the diploid were of the **a** mating type. Thus, HF5 has an *rme1* mutation. When the wild-type copy of *RME1* was transformed into HF5 (strain HF15), the DSB did not occur.

These results show that a haploid strain with an *rme1* mutation, when incubated in sporulation medium, will initiate meiotic recombination at the *HIS4* hot spot in a manner similar to that seen in a diploid strain. Thus, the formation of a DSB at a recombination hot spot does not appear to require pairing between homologous chromosomes. The level of DSB in the haploid strain, however, was lower than that observed in the related diploid strain (8 to 10% in HF5 compared with 19% in the diploid FX4). We do not know whether this difference is a consequence of a smaller fraction of cells entering the meiotic pathway in the haploid strain or an effect of the interaction between homologous chromosomes in the diploid.

DISCUSSION

The main results of this study are as follows. (i) The meiotic recombination hot spot at *HIS4* is associated with a meiosis-specific DSB; mutations in *cis* or *trans* that eliminate hot spot activity also eliminate the DSB. (ii) Basal levels of recombination at *HIS4* do not involve a DSB at the normal position. (iii) Insertions of telomeric sequences or multiple Rap1p binding sites upstream of *HIS4* (or *ARG4*) that stimulate meiotic recombination also stimulate formation of a DSB. (iv) The site at which the DSB occurs is not likely to represent a rigidly conserved recognition sequence. (v) A haploid strain with an *rme1* mutation, when incubated in sporulation medium, can form a DSB at the *HIS4* hot spot. Below, we discuss the implications of these results and compare our results with those of other workers.

DSBs have previously been associated with recombination in *S. cerevisiae* in several ways. In one study, a pattern of nonrandom chromosomal DSBs was examined on chromosomes III and IV (16), and two other studies correlated chromosomal regions with high levels of meiotic recombination to genomic regions with high levels of DSBs (48, 49). In addition, single recombination hot spots were associated with high levels of DSB formation (7, 9, 39). At the *ARG4* locus, Sun et al. (39) and De Massy and Nicolas (9) showed that deletions of sequences upstream of *ARG4* that reduce the frequency of recombination, in general, also reduce the frequency of DSBs. These studies indicated that the *ARG4* region located between positions −316 and −56 was required for formation of a DSB located near position −200. In one strain with a deletion (ΔHA315, positions −316 to −2) of the region thought to be essential for DSB formation, a meiotic DSB was detected, indicating that the rules for DSB formation at this locus are not simple (9). In addition, although there was a good correlation between the level of DSB and the frequency of gene conversion for most deletion derivatives, strains with the ΔHA315 deletion had reduced levels of DSB but normal levels of gene conversion.

There are two types of meiotic recombination at *HIS4*. The basal level of *HIS4* recombination is the level of exchange observed in strains with a deletion of the region upstream of *HIS4* (12), because this deletion removes the gradient of gene conversion normally observed at this locus. The same basal level is observed in strains with mutations in the Rap1p binding site upstream of *HIS4* or mutations in the transcription factors Bas1p and Bas2p (45, 46). In our strains sporulated at 25°C the basal level of aberrant segregation of markers located about 500 bp from the 5′ end of *HIS4* is 11 to 13%. The frequency of aberrant segregation for the same markers in strains with wild-type hot spot activity is about 19 to 23%. Thus, about half of the recombination frequency in wild-type strains is hot spot dependent and half is hot spot independent. When strains are sporulated at 18°C, a greater proportion of the recombination events appear to be hot spot dependent (15 to 18% basal level of aberrant segregation, 44 to 50% aberrant segregation in wild-type strains). In strains in which the wild-type *HIS4* upstream sequences are replaced with sequences with multiple Rap1p binding sites, enhanced levels of segregation are observed (53 to 62% at 25°C).

If we define the level of hot spot-dependent recombination at *HIS4* in our strains as the frequency of aberrant segregation greater than 11 to 12%, then the frequency of hot spot-dependent recombination is proportional to the frequency of the DSB (Fig. 3). In addition to this proportionality, the absolute frequency of DSBs is approximately that expected if the hot spot-dependent recombination is initiated by this type of DNA lesion. For example, the amount of DSB in wild-type strains is 3 to 5% and the amount of hot spot-dependent recombination (measured at a site about 500 bp from the DSB) is about 11%. Assuming that all recombination events are initiated by a DSB on one of the four chromatids, one would calculate that a recombination rate of 11% requires a 3 to 4% level of DSBs. The amount of DSB in MW154 (strain with telomeric insertions replacing the normal upstream sequences) is 19%, somewhat higher than that calculated by dividing the hot spot-dependent recombination frequency (50%) by 4. One reason for this discrepancy is that a significant fraction (about 30% [Table 3]) of the hot spot-dependent events in MW154 represent tetrads with two recombination events (aberrant 4:4, etc.). When a correction is made for these classes of tetrads, the predicted level of DSB (16%) is closer to the observed frequency.

Although these calculations suggest that most hot spot-dependent recombination is a consequence of a DSB, there are three additional factors that have not been taken into account. First, we previously showed that, although the *HIS4* hot spot stimulates recombination bidirectionally, most single recombination events involve either *HIS4* or *BIK1* (32). This result suggests that half of the DSBs should not contribute to the level of aberrant segregation at *HIS4*. Second, we do not know what fraction of the DSBs that are initiated in the upstream region include the genetic markers used in the experiment, which are usually located about 500 bp away. Because our previous study indicated that hot spot-dependent heteroduplexes usually extend to the end of *HIS4* (10), it is likely that most of the events processed toward *HIS4* include the marker. Third, we do not know the fraction of *rad50S* cells that undergo early meiotic events (DSB formation); in the isogenic *RAD50* derivatives, about half of the cells from tetrads (26). One possible explanation of the good agreement between the observed and expected levels of DSBs despite these factors is that only half of the *rad50S* cells undergo early meiotic events, which compensates for the effects predicted from the first factor discussed above.

Although hot spot-induced recombination events appear to be initiated by a DSB, no clear DSB is associated with the basal level of recombination at *HIS4*. There are several possible explanations for this result. First, the recombination events could be initiated from a DSB located outside of the *Bgl*II fragment used in most of our studies. Second, the event could be initiated from multiple weak sites of DSB within the *Bgl*II fragment. As mentioned previously, a very weak DSB in the *HIS4* gene is observed in one strain lacking hot spot activity (FX2); weaker sites would be undetectable. Third, the basal level of recombination could be initiated by a DNA lesion other than a DSB (for example, a single-strand nick). These possibilities might be resolved by studies of DSBs in a more extensive region surrounding *HIS4* and by examining DNA lesions with more sensitive PCR-based assays.

The insertion of telomeric sequences upstream of *ARG4* activates two new sites for DSB formation. The simplest interpretation of this result is that the position of the DSB is not solely a function of DNA sequence but involves an interaction between the DSB-inducing endonuclease and other DNA binding proteins. Such an interaction is supported by a number of other arguments. First, deletion of transcription factors that bind to the *HIS4* upstream region eliminates hot spot activity and DSB formation. Second, DSB formation occurs at approximately the same place at *ARG4* when different sequences are substituted for the wild-type sequences at the DSB site (9). Third, the strength of a DSB site can be affected by the context of that site in the chromosome (47). Fourth, the positions of

DSB sites are often correlated with the positions of sites in chromatin that are hypersensitive to DNase I (48).

Our observations and those of others suggest several possible explanations for the positioning and intensity of the DSB at *HIS4*. One possibility is that the position and intensity of the break reflect the degree to which DNA is "naked" in the chromatin. Alternatively, the DSB site could be determined by the degree to which DNA is bent by DNA-binding proteins. A third possibility for the *HIS4* site is that the position of the break is determined by a specific interaction between Rap1p and the DSB-forming endonuclease; in support of this possibility, several of the DSB sites at *HIS4* (in wild-type strains and strains with telomeric insertions) and *ARG4* (in strains with telomeric insertions) are located very close to the Rap1p binding site. It should be pointed out that an interaction between Rap1p and the DSB-forming nuclease cannot be a universal requirement for DSB formation, because there is no Rap1p binding site at the wild-type *ARG4* hot spot. A final possibility is that certain DNA-binding proteins result in attachment of the chromatin to a region of the cell in which meiotic recombination occurs. In this regard, it is of interest that Rap1p localizes to the nuclear periphery (22).

One surprising result of this study was that one of the two haploid parental strains formed a DSB when incubated in sporulation medium. Complementation analysis showed that the haploid with the DSB contained a naturally occurring *rme1* mutation, a mutation that allows the early events of meiosis without the requirement for expressed **a** and α mating-type information (20, 21). Previously, several other researchers (8, 17, 33) have detected DSBs in haploid strains expressing both **a** and α information, and De Massey et al. (8) have observed DSBs in *rme1* haploid strains. This result indicates that DSB formation does not require an interaction involving homologous chromosomes.

In summary, the level of hot spot-induced recombination at *HIS4* is proportional to the level of a meiosis-specific DSB located in the region upstream of *HIS4*. The hot spot-associated DSB can be eliminated by deletions that remove the normal region upstream of *HIS4* or by mutations in genes encoding transcription factors that bind to the *HIS4* upstream region. Our results and those of others indicate that the position and strength of the DSB are controlled by interactions (direct or indirect) between the DSB-inducing endonuclease and DNA-binding proteins.

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REFERENCES

1. **Alani, E., R. Padmore, and N. Kleckner.** 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:**419–436.
2. **Arndt, K., and G. R. Fink.** 1986. GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5′ TGACTC 3′ sequences. Proc. Natl. Acad. Sci. USA **83:**8516–8520.
3. **Arndt, K. T., C. Styles, and G. R. Fink.** 1987. Multiple global regulators control *HIS4* transcription in yeast. Science **237:**874–880.
4. **Becker, D. M., and L. Guarente.** 1991. High-efficiency transformation of yeast by electroporation. Methods Enzymol. **194:**182–187.
5. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.
6. **Buchman, A. R., W. J. Kimmerly, J. Rine, and R. D. Kornberg.** 1988. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**210–225.
7. **Cao, L., E. Alani, and N. Kleckner.** 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:**1089–1101.
8. **De Massy, B., F. Baudet, and A. Nicolas.** 1994. Initiation of recombination in *Saccharomyces cerevisiae* haploid meiosis. Proc. Natl. Acad. Sci. USA **91:** 11929–11933.
9. **De Massy, B., and A. Nicolas.** 1993. The control in *cis* of the position and amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae*. EMBO J. **12:**1459–1466.
10. **Detloff, P., and T. D. Petes.** 1991. Measurements of excision repair tracts formed during meiotic recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**1805–1814.
11. **Detloff, P., J. Sieber, and T. D. Petes.** 1991. Repair of specific base pair mismatches formed during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**737–745.
12. **Detloff, P. J., M. A. White, and T. D. Petes.** 1992. Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. Genetics **132:**113–123.
13. **Devlin, C., K. Tice-Baldwin, D. Shore, and K. T. Arndt.** 1991. RAP1 is required for BAS1/BAS2- and GCN4-dependent transcription of the yeast *HIS4* gene. Mol. Cell. Biol. **11:**3642–3651.
14. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1981. Mechanisms of gene conversion, p. 289–339. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Game, J. C.** 1993. DNA double-strand breaks and the *RAD50-RAD57* genes in *Saccharomyces*. Semin. Cancer Biol. **4:**73–83.
17. **Gilbertson, L., and F. Stahl.** 1994. Initiation of meiotic recombination is independent of interhomologue interactions. Proc. Natl. Acad. Sci. USA **91:**11934–11937.
18. **Goldway, M., A. Sherman, D. Zenvirth, T. Arbel, and G. Simchen.** 1993. A short chromosomal region with major roles in yeast chromosome III meiotic disjunction, recombination and double strand breaks. Genetics **133:**159–169.
19. **Goyon, C., and M. Lichten.** 1993. Timing of molecular events in meiosis in *Saccharomyces cerevisiae*: stable heteroduplex DNA is formed late in meiotic prophase. Mol. Cell. Biol. **13:**373–382.
20. **Hopper, A. K., and B. D. Hall.** 1975. Mating type and sporulation in yeast. I. Mutations which alter mating-type control over sporulation. Genetics **80:**41–59.
21. **Kassir, Y., and G. Simchen.** 1976. Regulation of mating and meiosis in yeast by the mating-type region. Genetics **82:**187–206.
22. **Klein, F., T. Laroche, M. E. Cardenas, F.-X. Hofmann, D. Schweizer, and S. M. Gasser.** 1992. Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast. J. Cell Biol. **117:**935–948.
23. **Malone, R. E., S. Kim, S. A. Bullard, S. Lundquist, L. Hutchings-Crow, S. Cramton, L. Lutfiyya, and J. Lee.** 1994. Analysis of a recombination hotspot for gene conversion occurring at the *HIS2* gene of *Saccharomyces cerevisiae*. Genetics **137:**5–18.
24. **Mitchell, A. P., and I. Herskowitz.** 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature (London) **319:**738–742.
25. **Nag, D. K., and T. D. Petes.** 1991. Seven-base-pair inverted repeats in DNA form stable hairpins *in vivo* in *Saccharomyces cerevisiae*. Genetics **129:**669–673.
26. **Nag, D. K., and T. D. Petes.** 1993. Physical detection of heteroduplexes during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**2324–2331.
27. **Nag, D. K., M. A. White, and T. D. Petes.** 1989. Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature (London) **340:** 318–320.
28. **Nicolas, A., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. Identification of an initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature (London) **338:**35–39.
29. **Perkins, D. D.** 1949. Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:**607–626.
30. **Petes, T. D., R. E. Malone, and L. S. Symington.** 1991. Recombination in yeast, p. 407–521. *In* J. R. Broach, E. W. Jones, and J. R. Pringle (ed.), The molecular and cellular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Ponticelli, A. S., E. P. Sena, and G. R. Smith.** 1988. Genetic and physical analysis of the M26 recombination hotspot of *Schizosaccharomyces pombe*. Genetics **119:**491–497.
32. **Porter, S. E., M. A. White, and T. D. Petes.** 1993. Genetic evidence that the meiotic recombination hotspot at the *HIS4* locus of *Saccharomyces cerevisiae* does not represent a site for a symmetrically processed double-strand break. Genetics **134:**5–19.

33. **Rockmill, B., and S. Roeder (Yale University).** 1994. Personal communication.
34. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.
35. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76:**4951–4955.
36. **Schultes, N. P., and J. W. Szostak.** 1990. Decreasing gradients of gene conversion on both sides of the initiation site for meiotic recombination at the *ARG4* locus in yeast. Genetics **126:**813–822.
37. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Stapleton, A., and T. D. Petes.** 1991. The Tn3 β-lactamase gene acts as a hotspot for meiotic recombination in yeast. Genetics **127:**39–51.
39. **Sun, H., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. Double-strand breaks at an initiation site for meiotic gene conversion. Nature (London) **338:**87–90.
40. **Sun, H., D. Treco, and J. W. Szostak.** 1991. Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **22:**1155–1161.
41. **Symington, L., A. Brown, S. G. Oliver, P. Greenwell, and T. D. Petes.** 1991. Genetic analysis of a meiotic recombination hotspot on chromosome III of *Saccharomyces cerevisiae*. Genetics **128:**717–727.
42. **Szankasi, P., W. D. Heyer, P. Schuchert, and J. Kohli.** 1988. Sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*: wildtype and mutant alleles including the recombination hotspot allele *ade6-M26*. J. Mol. Biol. **204:**917–925.
43. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double strand-break model for recombination. Cell **33:**25–35.
44. **White, M. A., P. J. Detloff, M. Strand, and T. D. Petes.** 1992. A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the *HIS4* locus in yeast. Curr. Genet. **21:**109–116.
45. **White, M. A., M. Dominska, and T. D. Petes.** 1993. Transcription factors are required for the meiotic recombination hotspot at the *HIS4* locus in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:**6621–6625.
46. **White, M. A., M. Wierdl, P. Detloff, and T. D. Petes.** 1991. DNA binding protein RAP1 stimulates meiotic recombination at the *HIS4* locus in yeast. Proc. Natl. Acad. Sci. USA **88:**9755–9759.
47. **Wu, T. C., and M. Lichten.** 1993. Position effects in meiotic recombination, p. 19–36. *In* F. P. Haseltine and S. Heyner (ed.), Meiosis II. American Association for the Advancement of Science Press, Washington, D.C.
48. **Wu, T. C., and M. Lichten.** 1994. Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science **263:**515–518.
49. **Zenvirth, D., T. Arbel, A. Sherman, M. Goldway, S. Klein, and G. Simchen.** 1992. Multiple sites for double-strand breaks in whole meiotic chromosomes of *Saccharomyces cerevisiae*. EMBO J. **11:**3441–3447.