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Genetics

Double strand breaks at the *HIS2* recombination hot spot in *Saccharomyces cerevisiae*

(meiosis/chromosomes/gene conversion/pairing)

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ABSTRACT **Double strand breaks (DSBs) have been found at several meiotic recombination hot spots in *Saccharomyces cerevisiae*; more global studies have found that they occur at many places along several yeast chromosomes during meiosis. Indeed, the number of breaks found is consistent with the number of recombination events predicted from the genetic map. We have previously demonstrated that the *HIS2* gene is a recombination hot spot, exhibiting a high frequency of gene conversion and associated crossing over. This paper shows that DSBs occur in meiosis at a site in the coding region and at a site downstream of the *HIS2* gene and that the DSBs are dependent upon genes required for recombination. The frequency of DSBs at *HIS2* increases when the gene conversion frequency is increased by alterations in the DNA around *HIS2*, and *vice versa*. A deletion that increases both DSBs and conversion can stimulate both when heterozygous; that is, it is semidominant and acts to stimulate DSBs in trans. These data are consistent with the view that homologous chromosomes associate with each other before the formation of the DSBs.**

The mechanism whereby homologous chromosomes find each other during meiosis and initiate recombination has been the source of considerable speculation and experimentation. Several models have been proposed to account for the genetic and, more recently, molecular data that have been obtained about meiotic recombination. The classical Holliday model (1) suggested that nicks are made in strands of the same polarity in two homologs, after which the broken strands are exchanged from one duplex to the other. The Meselson–Radding model (2) begins recombination with a single strand nick on one homolog, after which one end of the broken strand invades the homologous duplex. More recently, the double strand break (DSB) model for recombination was proposed by Szostak *et al.* (3); in this scheme, both ends of the broken duplex invade the homolog to initiate recombination. In the latter two models, one can imagine that the mechanism whereby homologs find each other involves *recA*-like (4) synaptase-assisted searching by the single strands created at the initiating breaks or nicks.

Since the publication of the DSB model, experiments have been done that demonstrate that DSBs occur during meiosis in yeast and that they are associated with recombination. These experiments may be sorted into two groups: (*i*) experiments that examine recombination hot spots both for recombination and DSBs, and (*ii*) experiments that look at the more global distribution of DSBs along entire chromosomes. Within the first class of experiments, Sun *et al.* (5) first demonstrated that a meiotic DSB occurs at the *ARG4* locus. The location of the DSB was in the DNA sequences that had been shown to be necessary (6) for conversion at *ARG4*. Furthermore, the time at which the breaks appeared was consistent with the time at which commitment to recombination occurred. In these early experiments, the DSBs were difficult to detect, in part because they were transient, as would be expected for an intermediate in the recombination process. This problem was solved by the discovery of a particular allele of the recombination gene *RAD50* (7). This allele (*rad50S*) allows DSBs to form, but prevents processing; thus, DSBs accumulate in meiosis, allowing them to be quantified. Cao, Alani, and Kleckner (8) demonstrated that DSBs occurring at an artificial hot spot (created by the fusion of the *HIS4* and *LEU2* genes) had the same general kinetics of appearance as DSBs at *ARG4*. In addition, DSB loci were eliminated by null mutations in either of the early recombination genes *SPO11* or *RAD50* (8). Thus, the DSBs occurring at both the *ARG4* and *HIS4*::*LEU2* hot spots exhibited the properties expected if they were involved in the initiation of recombination as predicted by the DSB model (3). More recently, meiotic DSBs have been found at the *HIS4* gene in strains that exhibit very high frequencies of recombination at this locus (9). Furthermore, these authors have shown a linear relationship between the amount of conversion and the amount of DSBs, leading them to suggest that DSBs initiate recombination at *HIS4*.

Not only did these known recombination hot spots have meiotic DSBs, but several studies have shown that DSBs occur in meiosis along many *Saccharomyces cerevisiae* chromosomes (10–12). Pulse field gel analysis demonstrates that breaks occur in meiosis at discrete locations. The numbers and locations of DSBs were generally consistent with known genetic map distances, compatible with the hypothesis that DSBs are involved in meiotic recombination.

We have been studying meiotic recombination at the *HIS2* gene in *S. cerevisiae.* This gene has a gradient of gene conversion that is high at the 3′ end and low at the 5′ end of the gene (13, 14); this polarity is opposite to that found in the well-studied *ARG4* and *HIS4* genes (6, 9). Consistent with the 3′ to 5′ gradient, the promoter region of the *HIS2* gene is not required for the high frequency of recombination observed (13, 14). In fact, at least some of the sequences necessary for high frequencies of conversion at *HIS2* appear to be located some 700 bp downstream of the coding region (14). The experiments in this paper demonstrate that there are meiosis-specific DSBs at *HIS2* and determine their properties.

MATERIALS AND METHODS

Yeast and Bacterial Strains. All yeast strains used in these experiments are derived from RM96-15AX (*MAT***a** *his2-xho ura3-13 met2-1 trp1-1*) and RM182-55C (*MATα cly3 cdc14-1 lys2-1 ade5 ade2-1 can1*^{r}). The diploid made by crossing RM96-15AX and RM182-55C is RM169. Mutations in the *HIS2* gene or other alterations (e.g., *rad50S*) were introduced by transformation as described below. The genotype of each strain at *HIS2* (and at *RAD50*, if appropriate) is shown in the appropriate table or figure. Two types of diploids were used. "Isogenic" diploids were made by directly crossing appropriate transformants of RM96-15AX and RM182-55C. "Congenic" diploids were made as

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Abbreviations: DSB, double strand break; ORF, open reading frame.
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follows. (*i*) A transformant of either RM96-15AX or RM182-55C (called P1) was crossed with the other parent (P2). (*ii*) A segregant from this cross with the appropriate genotype was then backcrossed at least three times with the P1 parent. (*iii*) A final segregant (which should be at least 94% identical to the P1 parent) was then crossed to the appropriate P2 parent to make the diploid. Each diploid used is denoted in the appropriate figure or table as isogenic or congenic. All tetrads dissected to determine the frequency of gene conversion at the *HIS2* locus were checked for false tetrads by examining the segregation of at least five other loci. The bacterial strain DH5α (Bethesda Research Laboratories) was used for all DNA manipulations.

Mutations in the *HIS2* Gene. All mutations in the *HIS2* region were made *in vitro* and placed in yeast by two-step gene replacement (15). The new mutation (*his2-hpa2*) used in this paper was made using oligonucleotide-directed mutagenesis as detailed in ref. 14. *his2-hpa2* is the second mutation we created at this *Hpa*I site, hence the *-hpa2* extension. The *his2-hpa2* mutation is a single-base pair substitution (A → T) at position +947, which ablates the *Hpa*I site and creates a stop codon. The *his2-2* mutation used to monitor heteroallelic recombination is located at +290 (14). A complete description of the construction of all other *his2* mutations, the Δ*Aha* deletion, and the TUBA66 insertion may be found in ref. 14. All mutations and alterations created *in vitro* and placed in yeast were confirmed by Southern blot analysis. A map of relevant loci is shown in Fig. 1.

Analysis of DSBs at *HIS2*. To study DSBs in and around the *HIS2* locus, the separation of function mutation *rad50S-KI81* (referred to henceforth as *rad50S*) was introduced by one-step gene replacement (15) as described in ref. 16 using the plasmid pNKY349 (provided by Nancy Kleckner, Harvard University). The *URA3* marker is inserted downstream of the *RAD50* coding region. All transformants were verified genetically and by Southern analysis as in ref. 16. To obtain cells in meiosis, diploids were grown to a concentration of 2×10^7 cells per ml in YPA, washed, and concentrated 2-fold in sporulation medium at 30°C. Media are defined in refs. 14 and 16. Samples (20 ml) were taken at the indicated times, and the cells were mixed with equal volumes of 95% ethanol at 4°C and 50 mM EDTA and stored at −20°C. To verify the progression of the cells in meiosis, aliquots were taken at each timepoint and counted for sporulation and 4′,6-diamidino-2-phenylindole (DAPI)-stained (7) to check the fraction of multinucleate cells. DNA was made as described in ref. 8. Gels were run at 50 V for 33–38 hr in TBE buffer in a 0.8% agarose gel at 4°C, as described (16, 17). All imaging and quantitation was done using a Molecular Dynamics PhosphorImager model 445SI as per instructions of the manufacturer.

RESULTS

The Gene Conversion Gradient at the *HIS2* Locus. All crosses in this paper were examined for five markers that were heterozygous, in addition to *HIS2* (see *Materials and Methods*). There were no significant differences in the frequencies of conversion of the other markers in diploids with or without alterations in or near the *HIS2* gene (data not shown). We have previously demonstrated that the polarity gradient of conversion in the *HIS2* gene is 3′ → 5′ (13, 14). For example, a single base mutation that created a *Nsi*I site (+6) had a gene conversion frequency of 4.9%, and a 4-bp insertion mutation at the *Xho*I site (+749) converted with a frequency of 14.1% (Fig. 1). Interestingly, an 8-bp linker insertion mutation located further 3′ at the *Hpa*I site (+947) converted at a lower frequency of 11.7% (14). In previous work (14), we suggested that the lower frequency observed at the *Hpa*I site was due to marker effects of the insertion. An alternative possibility was that the gradient reached its peak within the *HIS2* gene near the *Xho*I site and declined toward the *Hpa*I site. We constructed a single base change mutation in the *Hpa*I site (see *Materials and Methods*); the frequency of conversion is 20.7% (62 conversions in 299 tetrads). Thus, the frequency of conversion appears to increase all the way across the *HIS2* gene (see *Discussion*).

DSBs at the *HIS2* Locus. To determine whether DSBs occur during meiosis in the *HIS2* region, we constructed *rad50S* strains (8). The data in Fig. 2 indicate that two DSBs occur in the *HIS2* region, and they appear with the kinetics that would be expected if they were associated with recombination. The location of the breaks is consistent with the gene conversion polarity gradient (see *Discussion*). We have labeled the break in the coding region (at position 650 ± 30) as break C and the one 200-bp downstream of the coding region (at position 1200 ± 30) as break B. To compare the time at which DSBs appear to the time of commitment to recombination, we measured the appearance of His^+ recombinants in a *RAD50* derivative of the *rad50S* diploid (Fig. 3). From these results, it appears that the DSBs at *HIS2* appear at a time consistent with their being involved in meiotic recombination. However, we note that the experiments have to be done in two different strains (*rad50S* and *RAD50*), and it is possible that the timing of their progression through meiosis varies. Finally, we examined the effect of a mutation in an early Rec gene (*REC104*) on the DSBs. A *rec104* mutation eliminates all meiotic recombination (18) and, as Fig. 2 demonstrates, DSBs at *HIS2* cannot be detected in the *rec104* mutant. We have obtained the same results with *rec102* and *rec114* mutations (data not shown). We conclude that the DSBs found at the *HIS2* locus have many of the properties of meiotic DSBs found at other *S. cerevisiae* recombination hot spots.

Relationship of DSBs and Gene Conversion. Our previous work has demonstrated that sequences necessary for the high frequency of conversion at *HIS2* are located some 700 bp downstream of the coding region (i.e., downstream of position +1701; ref. 14). The separation between the sites where the breaks occur and sequences necessary for conversion provided an opportunity to ask how conversion at *HIS2* is related to the DSBs observed. This separation allowed us to alter the frequency of conversion without altering the sequences near the DSB sites. In our previous studies of conversion at *HIS2*, we examined a deletion (Δ*Aha*) of 382 bp from position +1349 to position +1731, and an insertion (TUBA66) of 5.5 kb at position +1701 (ref. 14; Fig. 1). Δ*Aha* increases gene conversion of the *his2-xho* allele from 14.1% to 23.8%, and the TUBA66 insertion decreases conversion from 14.1% to 2.2% (Table 1). [We note that the effects of both the Δ*Aha* and TUBA66 alterations are limited to the *HIS2* locus; neither alteration significantly changed gene conversion at any of five other loci (data not shown).] The data

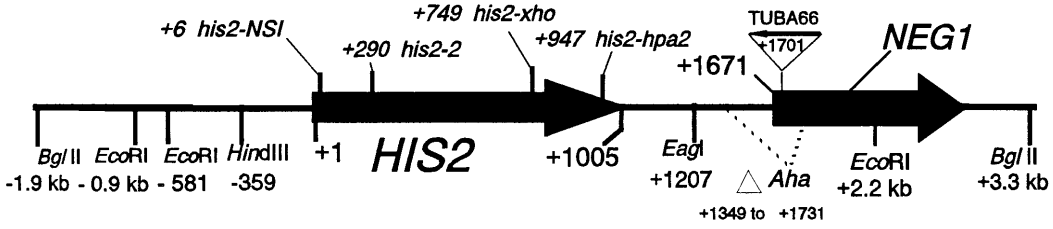

FIG. 1. Map of the *HIS2* gene and surrounding region. Features shown are those used in the experiments described in this paper. All locations are numbered relative to the A of the first ATG of the *HIS2* coding region (designated +1). The arrow indicates the direction of transcription. The location of the downstream gene (*NEG1*) is also shown.

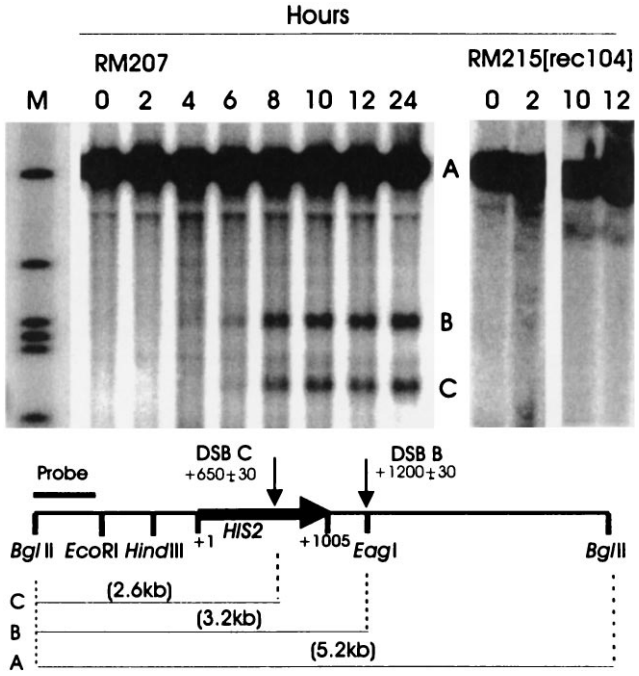

FIG. 2. Southern analysis of DSBs during meiosis at the *HIS2* locus. Cells were removed from sporulation medium at the times indicated, and DNA was made, digested with *Bgl*II, and analyzed as described. The numbers above the lanes refer to the time in meiosis. The parental band A and the DSB bands B and C are illustrated in the figure. The probe was the *Bgl*II–*Eco*RI fragment shown on the figure. The diploid used for the wild-type lanes was RM207 (*his2-xho/HIS2 rad50S/rad50S*, congenic to RM169). On the right side of the figure are four time points from a congenic *rec104-Δ1/rec104-Δ1 rad50S/rad50S* diploid (RM215). The lane labeled M contains markers of sizes 5.2 kb, 3.9 kb, 3.2 kb, 3.0 kb, 2.9 kb, and 1.9 kb, from top to bottom. All lanes shown were run on the same gel.

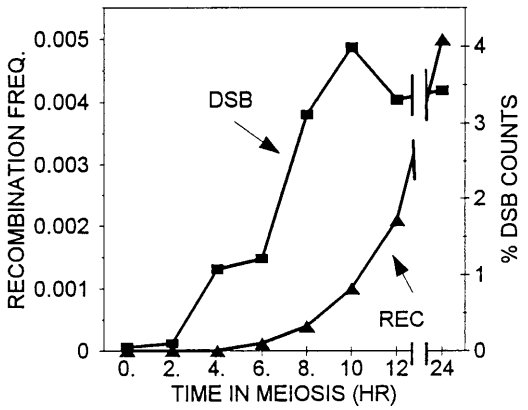

FIG. 3. Time course of commitment to recombination and DSB formation in meiosis at the *HIS2* locus. Recombination frequency is the number of His^+ recombinants divided by the total number of colonies. The percent DSB counts is the amount of counts found in the DSB bands (at time *t*) divided by the total number of counts in the parental and DSB bands (at time *t*). The time course of DSBs (■) was from one experiment using RM207 (*his2-xho/HIS2 rad50S/rad50S*, congenic to RM169; see also Table 1). The recombination values shown are the frequency of His^+ prototrophs (▲) in a return to growth experiment in a congenic diploid of genotype *his2-xho/his2-2 RAD50/RAD50*.

in Fig. 4 show that the presence of Δ*Aha* increases the amount of both DSB bands, but does not change their position or the kinetics of their appearance (see *Discussion*). For example, in the experiment shown in Fig. 4*B*, DSB B, located at +1200, is increased from the 10- and 12-hr average of 1.8% to 4.0%, and DSB C, at +630, is increased from 2.5% to 9.1%. In two independent experiments, we could not detect (<0.5%) any DSB bands in the *HIS2* region in the diploid containing the TUBA66 insertion (data not shown). Thus, there is a correlation, although not a linear one, between the amount of conversion and the amount of DSBs at *HIS2* observed during meiosis (Table 1; see *Discussion*).

Analysis of Chromosome Preference for DSBs in Strains Heterozygous for Δ*Aha*. The data above indicate that there is 1.7 times as much gene conversion and 2.8 times as many DSBs in diploids homozygous for Δ*Aha* as there are in normal strains (Table 1). If the *Aha* deletion were capable of stimulating DSBs and recombination only on the chromosome in which it was present, then recombination in a strain heterozygous for Δ*Aha* should have both a physical and a genetic consequence. First, more DSBs should occur on the homolog containing Δ*Aha* (the "hot" chromosome) than on the chromosome without Δ*Aha* (the "cold" chromosome). Second, the DSB model suggests that there should be a loss of parity in gene conversion; more recombination should initiate on the hot chromosome, leading to preferential loss of the marker on that chromosome (19). The data in Table 2 demonstrate no significant disparity in four independent experiments with diploids heterozygous for Δ*Aha*. We also note that the overall frequency of gene conversion in all diploids heterozygous for Δ*Aha* was significantly (95% confidence level; *G* test) elevated over RM169 (Table 2). This indicates that the presence of the *Aha* deletion stimulates recombination even when heterozygous (see *Discussion*).

To test the first prediction made above, we examined DSBs in diploids heterozygous for Δ*Aha* that were homozygous for the *rad50S* allele. If the *Aha* deletion were capable of stimulating recombination only in the chromosome on which it was located, there should be 2.8 times more breaks on the hot chromosome. To differentially detect breaks on the hot versus the cold chromosome, we used a probe located downstream of the deletion (i.e, the *Eco*RI–*Bgl*II fragment shown to the right in Fig. 1). [When

Table 1. Correlation between gene conversion levels and the amount of DSBs

Gene conversion				DSB measurements			
Diploid	Genotype	No. of tetrads	GC content, %	Diploid	Genotype	No. of experiments	DSB, %
RM169	*his2-xho* / *HIS2*	391	14.1	RM207	*his2-xho* / *HIS2* *rad50S* / *rad50S*	5	4.3 ± 0.8
RM198	*his2-xho-ΔAha* / *HIS2-ΔAha*	319	23.8	RM206	*his2-xho-ΔAha* / *HIS2-ΔAha* *rad50S* / *rad50S*	3	12.2 ± 0.6
RM187	*his2-xho::TUBA66* / *HIS2::TUBA66*	316	2.2	RM216	*his2-xho::TUBA66* / *HIS2::TUBA66* *rad50S* / *rad50S*	2	<0.5*

The percent DSB = (the counts in the DSB bands divided by the total number of counts) × 100. RM207(*rad50S/rad50S*) is congenic to RM169; RM206 (*rad50S/rad50S*) is congenic to RM198; and RM216 (*rad50S/rad50S*) is congenic to RM187. DSBs were measured at 10 and 12 hr. The DSB values presented are the average of the number of experiments shown.

*No bands at the normal DSB positions were detected in two independent experiments. Reconstruction experiments indicate that bands consisting of 0.5% of the total DNA could be detected.

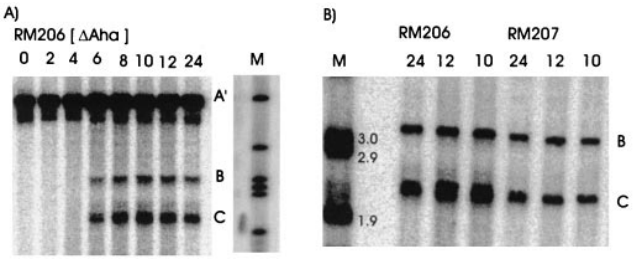

FIG. 4. Southern blot analysis of DSBs in a diploid homozygous for Δ*Aha*. (*A*) The diploid RM206 is homozygous for Δ*Aha* and the *rad50S* mutation and is congenic to RM169. Cells were removed from sporulation medium at the time noted at the top of each lane. Markers (M) shown are 5.2 kb, 3.9 kb, 3.2 kb, 3.0 kb, 2.9 kb, and 1.9 kb, from top to bottom. Labels and the probe are as in Fig. 2 except band A′ is the parental band from RM206 and is 382 bp smaller than the normal (wild-type) band, which is designated as A (see Fig. 2). (*B*) Evidence that DSB bands from the Δ*Aha* diploid are the same size as the normal diploid. An enlargement of the DSB bands is shown. DNA from the times in meiosis indicated were analyzed as in *A*. RM206 is homozygous for Δ*Aha*; RM207 does not contain the deletion. The markers (lane M) in *B* are a subset of those used in *A*. [The total amount of counts in the bands A (not shown), B, and C (for RM207) was 1.3×10^6 at 10 hr, 1.4×10^6 at 12 hr, and 2.0×10^6 at 24 hr; the total amount of counts in bands A′ (not shown), B, and C (for RM206) was 1.4×10^6 at 10 hr, 1.2×10^6 at 12 hr, and 1.5×10^6 at 24 hr.]

used in an experiment with either a normal or homozygous Δ*Aha* strain, the frequency and location of DSBs detected by this "downstream" probe were the same as those detected by the normal "upstream" probe (data not shown).] Thus, in Fig. 5, the DSB fragments generated from the two homologous chromosomes differ by 382 bp in length. The data in Fig. 5 have been quantitated in Table 3, along with two additional experiments. The data clearly indicate that breaks occur equally on both homologs. The percentage of total DNA broken in the four experiments averages 9.4%; this value is elevated over the average value of 4.3% found in the strain with no deletion (Table 1). Thus, as was found above for gene conversion, the hot chromosome with Δ*Aha* is capable of stimulating DSBs in the heterozygote; interestingly, it does so equally on both homologs.

DISCUSSION

In *S. cerevisiae*, several loci have been studied in detail both for gene conversion and for DSBs; the *ARG4* locus has, perhaps,

Table 2. Genetic analysis of heterozygous *Aha* deletion

Diploid	Genotype	Total no. of tetrads	GC, %	3:1/1:3 (parity)
RM169	*his2-xho* / *HIS2*	391	14.1	25/26 (0.96)
RM198	*his2-xho-ΔAha* / *HIS2-ΔAha*	319	23.8	33/41 (0.80)
RM212	*his2-xho-ΔAha* / *HIS2-ΔAha*	303	21.1	32/31 (1.03)
RM197	*his2-xho* / *HIS2-ΔAha*	274	22.6	29/30 (0.97)
RM211	*his2-xho* / *HIS2-ΔAha*	318	18.9	26/31 (0.84)
RM203	*his2-xho-ΔAha* / *HIS2*	296	23.6	36/31 (1.16)
RM213	*his2-xho-ΔAha* / *HIS2*	290	19.0	27/27 (1.00)

None of the parity values were significantly different from 1.0 using the χ^2 test at the 95% confidence level. In fact, the two most different values (RM198 and RM203) were not significantly different ($\chi^2 = 0.838$; $P = 0.36$). RM212, RM211, and RM213 are isogenic to RM169. RM198, RM197, and RM203 are congenic to RM169.

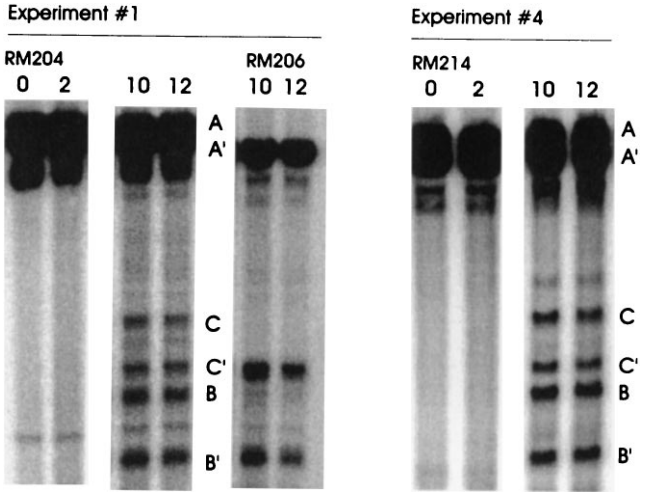

FIG. 5. Southern blot analysis of diploids heterozygous for Δ*Aha*. The experiments were done as in Fig. 2, except that the probe was the *Eco*RI–*Bgl*II fragment shown on the right of Fig. 1. Bands from the Δ*Aha* chromosome are A′, B′, and C′. Bands from the normal chromosome are denoted A, B, and C. Because the break bands are probed from the right, they include the deletion. The difference in sizes between the bands A and A′, B and B′, and C and C′ is 382 bp. The DNA from RM206 (homozygous for Δ*Aha*) was run on the same gel as the DNA from RM204 (heterozygous for Δ*Aha*); the positions of the bands in RM206 allow the four bands from RM204 to be assigned to the hot or cold chromosome. Both diploids were homozygous for *rad50S*. RM204 is congenic to RM169; RM214 is isogenic to RM169.

been examined in the most detail. At *ARG4*, several hundred base pairs of the promoter region are necessary, but not sufficient, for the normal levels of gene conversion observed at the gene (6, 20). The DSB observed in meiosis at this locus occurs in the same region (5). A number of laboratories have mapped meiotic DSBs in detail along whole chromosomes in *S. cerevisiae* and have found that breaks occur in regions between open reading frames (10–12). Given the close packing of yeast genes, these regions often contain promoters. Meiotic DSBs also tend to occur in regions that are more susceptible to DNase I cleavage; this observation is consistent with the hypothesis that DSBs occur in "open" chromatin (12). Since active promoters are often found in a more open configuration than other types of DNA, this also explains the association between DSB sites and promoter regions (12).

The recombination hot spot at the *HIS2* gene displays gene conversion frequencies as high as 20.7% at the *his2-hpa2* allele located ≈50 bp from the end of the coding region. The DSB model of Szostak *et al.* (3) predicts that the *HIS2* locus should exhibit a high level of DSBs. The data presented in this paper are consistent with that prediction. In the normal *HIS2* locus (with no alterations present), ≈4.3% of the DNA exhibits a DSB. The breaks appear early in meiosis and are dependent upon known recombination functions, and the amount of DSBs is correlated with the amount of gene conversion observed. If every DSB resulted in a detectable gene conversion event and if breaks occurred on only one of the four chromatids, one would expect a ratio of conversion/DSBs equal to 4. For the *-hpa2* allele located near the high end of the polarity gradient at *HIS2*, the ratio for the wild-type diploid containing no alterations is 20.7%/4.3% = 4.8. We note that the correlation between the amount of DSBs and the amount of gene conversion is not linear, unlike the correlation at *HIS4* (9). For the *his2-xho* allele measured (Table 1), the ratios of conversion to DSBs are >4.4, 3.3, and 1.95 for the TUBA66, wild-type, and ΔAha diploids, respectively. The efficiency of turning a DSB into a conversion event at *HIS2* appears to decrease as there are more breaks.

One of the things that is novel about DSBs and the *HIS2* hot spot is the locations of the breaks compared with the other natural

Table 3. DSBs in diploids heterozygous for the *Aha* deletion

Experiment (strain)	Chromosome broken		10 hr DSB, %	10 hr Ratio, hot/cold	12 hr DSB, %	12 hr Ratio, hot/cold	Average ratio hot/cold ± SD
1 (RM204)	Cold	B	2.67		2.61		
		C	1.99		2.02		
		(B+C)	4.66		4.63		
				1.09		1.03	1.06 ± 0.04
	Hot	B′	2.95		2.73		
		C′	2.11		2.05		
		(B′+C′)	5.06		4.78		
2 (RM204)	Cold	B	2.76		2.63		
		C	2.20		2.18		
		(B+C)	4.96		4.81		
				1.00		1.06	1.03 ± 0.04
	Hot	B′	2.72		2.76		
		C′	2.25		2.35		
		(B′+C′)	4.97		5.11		
3 (RM204)	Cold	B	2.28		2.02		
		C	1.70		1.60		
		(B+C)	3.98		3.62		
				0.92		1.04	0.98 ± 0.08
	Hot	B′	2.33		2.62		
		C′	1.34		1.16		
		(B′+C′)	3.67		3.78		
4 (RM214)	Cold	B	2.27		3.25		
		C	1.99		2.43		
		(B+C)	4.26		5.68		
				0.97		0.91	0.94 ± 0.04
	Hot	B′	2.30		3.07		
		C′	1.81		2.12		
		(B′+C′)	4.11		5.19		
Overall average				1.00 ± 0.07		1.01 ± 0.07	1.00 ± 0.2

RM204 has the genotype *his2-xho-ΔAha/his2-NSI rad50S/rad50S* and is congenic to RM169. RM214 has the genotype *his2-xho-ΔAha/HIS2 rad50S/rad50S* and is isogenic to RM169. The hot chromosome refers to the homolog containing the Aha deletion; the cold chromosome refers to the homolog without the Aha deletion. B, B′, C, and C′ are defined as in Fig. 5. The times refer to cells taken at 10 hr and 12 hr after introduction into sporulation medium. The ratio hot/cold represents the total percent of DSBs on the hot chromosome divided by the total percent on the cold chromosome. A hot/cold ratio of 1.0 indicates that DSBs were formed equally on both homologs.

yeast loci that have been studied both genetically and physically. DSB C actually occurs in the coding region of *HIS2*. This argues that meiotic DSBs can occur in the middle of a transcribed region. [We have demonstrated that the *HIS2* gene is transcribed in meiosis at approximately the same level as mitosis (data not shown).] Although most meiotic DSBs appear to be associated with intergenic regions, DSB C at *HIS2* indicates that there can be other possibilities. The data indicating a relationship between open chromatin and DSB sites (12) predict that the internal DSB C in the *HIS2* gene is a DNase I sensitive site. We are in the process of testing that prediction.

The downstream DSB B at *HIS2* occurs ≈200 bp 3′ of the coding region (Fig. 2; Fig. 6). The next open reading frame (ORF; YFR024C) downstream of *HIS2* starts at position +1671 (relative to the +1 of the *HIS2* ORF). [We have demonstrated that this ORF is not essential; neither deletions of it nor insertions in it have any effect on cell viability, growth rate, or meiosis (data not shown). We have therefore referred to this ORF as *NEG1*, for nonessential gene (Fig. 1).] *NEG1* is transcribed in both mitosis and meiosis (data not shown), and the direction of transcription is the same as *HIS2*. Thus, the *NEG1* promoter is located somewhere in the 665 bp of DNA between the last base of the *HIS2* ORF (+1005) and the first base of the *NEG1* ORF (+1671). There are possible TATA boxes at +1374 and +1238. We conclude that the downstream DSB B (at position +1200) could be located in the *NEG1* promoter. We have demonstrated previously (14) that alterations located >500 bp downstream of the putative *NEG1* promoter region can affect gene conversion at *HIS2*. This suggests that, if the *NEG1* promoter is necessary for the high frequency of conversion observed at *HIS2*, it is not sufficient. Consistent with this, moving the 5.2-kb *Bgl*II fragment (Fig. 1) to chromosome II did not retain the recombination hot spot (14), even though the *NEG1* promoter and ORF were present. To the extent that chromatin structure is important in determining DSB sites (12), these data suggest that such structure can be affected by factors other than local sequence. Finally, we note that the locations of the DSBs in strains with the *Aha* deletion (Fig. 4) are not detectably different from the locations in normal strains, even though the number of DSBs is increased 2.8-fold. This suggests that the locations of the breaks have some sequence specificity. If true, this indicates that the recombination hot spot at *HIS2* has at least two separable components: the sites where the DSBs actually occur and the downstream sequences we have demonstrated are necessary for both the DSBs and conversion (14).

The second novel property observed at *HIS2* is that the DSBs occur equally on both homologs, even when only one of the two chromosomes contains the Δ*Aha* alteration. DSB bands from both parents were examined simultaneously in one experiment, thereby allowing the breaks on the two chromosomes to be easily compared (Table 3). Since the hot chromosome stimulates breaks on the cold chromosome, Δ*Aha* formally can be defined as semidominant; said another way, the deletion acts to stimulate recombination in trans. In the original version of the DSB model (3), and in the various modifications of it that have been proposed since, it was proposed that recombination is initiated by the broken ends of one homolog invading the other. For example, it has been suggested that the interaction of the single-stranded tails

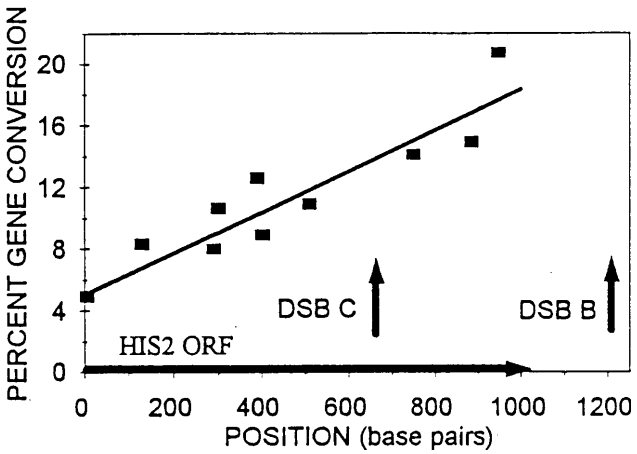

FIG. 6. Map of DSBs relative to conversion gradient. Data for conversion comes from ref. 14, except for the *his2-hpa2* allele. The arrow on the *x*-axis represents the *HIS2* coding region. The least squares line has a correlation coefficient of 0.85. Vertical arrows represent the position of the DSBs.

(from the DSB) with an intact homologous duplex helps to initiate recombination between homologs (21). More recently, Weiner and Kleckner (22) found evidence for interactions between homologs early during meiosis. These meiotic "interstitial" interactions were independent of the synaptonemal complex and did not require meiosis-specific DSBs. From these data, they proposed a model in which early meiotic pairing occurs by closely related DNA–DNA interactions between intact duplexes, possibly by paranemic interactions (22). They further hypothesized that meiotic DSBs converted these initial interactions from paranemic to plectonemic recombination intermediates. The Weiner and Kleckner model clearly proposes that DSBs occur after homologs are already interacting.

The features of the DSBs found at *HIS2* are absolutely consistent with the hypothesis that DSBs are involved in, and required for, recombination at *HIS2*, as has been found for other loci in yeast. The data from analysis of the heterozygous Δ*Aha* strains (Fig. 5; Table 3) are consistent with the view that meiotic DSBs are formed after homologs are already interacting, perhaps in the manner proposed by Weiner and Kleckner (22). If, in the diploid heterozygous for Δ*Aha*, DSBs occurred independently on each chromosome at frequencies similar to those found in the homozygotes, the hot chromosome containing Δ*Aha* should have 2.8 times as many breaks as the cold one. In contrast, the DSBs occur equally on both homologs. One could imagine that the hot chromosome is better at attracting the proteins involved in making the DSBs, but that once such proteins were present, they could make the breaks on either homolog with equal probability because the two chromosomes are in contact.

In an examination of DSBs occurring at the *HIS4*::*LEU2* hot spot (a hot spot created when 77 bp of *Escherichia coli* DNA were coincidentally inserted at the junction), Xu and Kleckner (23) found that inserting an additional 32-bp *Bam*HI linker increased the frequency of DSBs from 18.4% to 24.3% when homozygous; recombination frequencies were not reported. There are two breaks at the *HIS4*::*LEU2* hot spot, but only the one near the location of the inserted linker DNA was stimulated. The frequency of the other DSB was diminished by the insertion from 4.4% to 2.2%. Xu and Kleckner suggested that there might be competition between the two break sites (some 2 kb apart). We note that both *HIS2* DSBs reacted similarly to alterations that affected recombination; both breaks were increased by the Δ*Aha*, and both were decreased by the TUBA66 insertion. We do not see evidence for competition between the two DSBs (located ≈600 bp apart) at *HIS2*. This might reflect differences between natural and artificial hot spots, the closer distance between the breaks at *HIS2*, or our alterations not being located at the sites where the breaks occur. Xu and Kleckner (23) also examined DSBs in crosses heterozygous for the stimulating *Bam*HI linker. In such crosses, the total amount of DSBs increased to 20.7%, a value intermediate to those found in the homozygotes (see above). As in the crosses with the homozygous linker, the entire increase was in the site close to the linker. Although the differences were slight, the authors speculated that there was a trans effect of the *Bam*HI linker; they argued that its presence on the hot homolog decreased DSBs at both sites on the other cold homolog. If their suggestion proves to be true, then the trans effect of alterations at the *HIS2* locus and the *HIS4*::*LEU2* locus are in opposite directions. However, both observations are consistent with the view that homologs interact before DSBs are formed.

Finally, we note that at least 10 genes are required for the initiation of meiotic recombination in *S. cerevisiae* (16). Mutations in these "early exchange" genes [e.g., *REC104* (18)] reduce meiotic recombination to background mitotic levels; in those that have been tested, DSBs are not detectable (e.g., see Fig. 2). One can posit at least three types of roles for these early exchange genes. First, some of the functions encoded by these genes may act directly in the formation of DSBs. Second, some of them may be required both for the homolog interactions proposed by Weiner and Kleckner (22) and for formation of DSBs. Third, some of the early exchange functions might play a role only in pre-DSB homolog interaction. Weiner and Kleckner demonstrated that a *spo11* mutation (which prevents DSBs) reduced interstitial interactions to 10% of normal, consistent with either the second or the third idea. If the only role of one of the early exchange genes were to make interstitial interactions between homologs, this would imply that such interactions are required for recombination in diploids since mutations in the early exchange genes abolish interchromosomal meiotic recombination. However, two laboratories have found meiotic DSBs in haploids; they occur at the appropriate places and with nearly normal kinetics (24, 25). If the role of any of the early exchange genes were limited to the formation of interstitial interactions, one would predict that mutations in such genes would have little effect upon DSBs occurring in a haploid meiosis, since there are no homologs present. Of the early exchange genes, only the effect of a *rad50* mutation in haploid meiosis has been reported, and the *rad50* null mutation reduces the amount of DSBs that occur in a haploid meiosis (25).

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