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Relationship between Nuclease-Hypersensitive Sites and Meiotic Recombination Hot Spot Activity at the *HIS4* Locus of *Saccharomyces cerevisiae*

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Meiotic double-strand DNA breaks (DSBs), the lesions that initiate meiotic recombination at the *HIS4* recombination hot spot, occur in a region upstream of the coding sequence associated with multiple DNase I-hypersensitive sites. Mutations in transcription factors that lead to loss of the DSBs result in the loss of some but not all DNase I-hypersensitive sites in the upstream region. A meiosis-specific change in chromatin structure is detected in strains with the wild-type hot spot but not in strains with alterations that elevate or reduce hot spot activity. The position and intensity of micrococcal nuclease-hypersensitive sites correlate poorly with the sites of DSB formation.

Meiotic recombination events occur at high frequency in certain regions of the *Saccharomyces cerevisiae* genome (hot spots) (19). Hot spots have been localized near the *HIS4* (7, 29, 30), *ARG4* (6, 17, 26), and *HIS2* (15) loci. In addition, a duplicated segment of *LEU2* (4), a Tn*3*-derived transposable element (24), and a centromere-linked region of chromosome III (27) are meiotic recombination hot spots in *S. cerevisiae*. Both the *ARG4* and *HIS4* hot spots are located upstream of the coding sequences and are associated with meiosis-specific double-strand DNA breaks (DSBs) (7, 9, 17, 25). Thus, these hot spots appear to stimulate recombination because they represent sites that are particularly sensitive to the endonuclease that initiates meiotic recombination.

Two types of experiments demonstrate that hot spot activity requires a specific chromatin structure (interaction between DNA and DNA-binding proteins) rather than a specific DNA sequence. First, at the *HIS4* locus, hot spot activity requires the binding of three transcription factors, Rap1p, Bas1p, and Bas2p (Fig. 1) (9, 29, 30). The stimulation of recombination by these proteins is not directly related to transcription, since deletion of the *HIS4* TATAA sequence, which greatly reduces *HIS4* transcription, has no effect on hot spot activity (28). Second, in two yeast studies (18, 31), a correlation was observed between nuclease-hypersensitive regions in chromatin and hot spots for recombination. Wu and Lichten (31) found that DSB formation occurred in DNase I-hypersensitive regions of chromatin; the patterns of hypersensitive sites were similar in mitotic and meiotic cells. Ohta et al. (18) reported that hot spots for DSB formation occurred at sites that were hypersensitive to micrococcal nuclease (MNase) at the *ARG4* and *CYS3* loci. The sites associated with DSB formation were about threefold more sensitive to MNase in meiosis than in mitosis. In addition, Shenkar et al. (22) found DNase I-hypersensitive sites associated with the mouse Eβ recombination hot spot.

In previous studies, we constructed strains with various levels of hot spot activity at the *HIS4* locus and showed a correlation between the level of hot spot activity and the frequency of DSB formation at the hot spot (9). In this report, we examine the relationship between hot spot activity and sensitivity of the chromatin to DNase I and MNase. We find that hot spot-associated DSBs occur upstream of *HIS4* in same region as DNase I-hypersensitive sites but are not associated with MNase-hypersensitive sites. Deletion of the Bas1p or Bas2p transcription factor, which eliminates hot spot activity and DSB formation, results in loss of some but not all of the DNase I-hypersensitive sites in the *HIS4* upstream region. These results indicate that recombination hot spots require a factor(s) in addition to open chromatin, and we suggest that one such factor may be an interaction between transcription factors and the meiosis-specific endonuclease that catalyzes DSB formation.

MATERIALS AND METHODS

Plasmids. Two plasmids, pAK1 and pSP2, were used as hybridization probes. 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. Plasmid pSP2 (from S. Porter, University of North Carolina) had an *Xho*I fragment (containing *BIK1* and the 5′ third of *HIS4*) in the vector B142; B142 is a derivative of YIp5 that lacks the *Pvu*II site.

Yeast strains. All diploid yeast strains used in this study were derived by mating haploid derivatives of AS4 (α *trp1 arg4 tyr7 ade6 ura3*) and AS13 (**a** *leu2 ade6 ura3*). These derivatives contained alterations (introduced by transformation) at the *HIS4* locus or in transcription factors associated with *HIS4* transcription. The relevant genotypes of the diploids and references for their construction are given in Table 1. DSBs were monitored in diploids homozygous for the *rad50S* mutation (Table 1).

Media and genetic techniques. Standard procedures for mating and transformation were used (23). Liquid sporulation at 25°C was performed as described previously (9).

Analysis of DNase I-hypersensitive sites in chromatin. The preparation of cell lysates and DNase I digestion of chromatin were done according to the method of Wu and Lichten (31). Cells (100 ml) at a density of 2×10^7/ml were collected for each isolation. Spheroplasts were prepared by incubation of cells in spheroplasting buffer (1.0 mM sorbitol, 25 mM potassium phosphate, 25 mM sodium succinate, 10 mM magnesium chloride, 10 mM β-mercaptoethanol [pH 5.5]) containing 10 mg of Zymolyase 20 T at 30°C for 5 min. The spheroplasts were then washed with spheroplasting buffer plus protease inhibitors (10 mM sodium cacodylate, 2 mM iodoacetate, 0.2 μM aprotinin, 130 μM bestatin, 1 μM leupeptin, 1 μM pepstatin) and then lysed in 10 ml of 100 mM sucrose–10 mM Tris-HCl (pH 7.5)–140 mM sodium chloride–1 mM magnesium chloride–0.2% Nonidet P-40–0.5 mM phenylmethylsulfonyl fluoride (PMSF)–the protease inhibitor mix described above. The lysate pellets were resuspended in 1.2 ml of digestion buffer (100 mM sucrose, 10 mM Tris-HCl [pH 7.5], 140 mM sodium chloride, 2.5 mM calcium chloride, 3.5 mM magnesium chloride). Each digestion was performed on ice for 5 min in a 400-μl volume with 0, 50, or 100 U of DNase I (Promega). Control digests with naked DNA were done with 10 μg of DNA in a 400-μl volume with 0, 10, or 20 U of DNase I.

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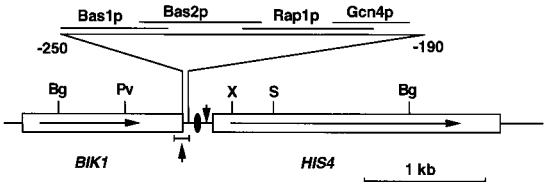

FIG. 1. Physical maps of the wild-type *HIS4* and *BIK1* loci (based partly on data derived from references 1, 2, and 8). Coding sequences are indicated by rectangles, and directions of transcription are indicated by arrows within the rectangles. The TATAA sequence is represented by a black oval. The vertical arrow above the map shows the transcription start, and the vertical arrow below the map indicates the position of the DSB (9). The bracketed region is the sequence replaced by oligonucleotides containing various protein binding sites in some yeast strains with altered hot spot activity. The expanded region above the linear map represents protein binding sites for the wild-type hot spot. The numbers associated with the expanded region represent the distance to the initiation codon of *HIS4*. The *Bgl*II-*Pvu*II fragment of *BIK1* was used as a hybridization probe in the chromatin studies. Mutations used to monitor aberrant segregation were located at the *Sal*I site. Abbreviations for restriction enzymes: Pv, *Pvu*II; Bg, *Bgl*II; S, *Sal*I; RV, *Eco*RV.

DNase I treatment was terminated by adding EDTA to a concentration of 12.5 mM and sodium dodecyl sulfate (SDS) to a concentration of 0.5%. DNA was then extracted twice with phenol-chloroform-isoamyl alcohol and once with chloroform-isoamyl alcohol. Isolated DNA was treated with RNase A and extracted once with chloroform-isoamyl alcohol. The DNA samples were then precipitated with ethanol, washed with 70% ethanol, and resuspended in the *Bgl*II digestion buffer recommended by New England Biolabs. The resulting DNA fragments were separated on a 0.8% agarose gel. Standard Southern analysis was performed. To examine the *HIS4* recombination hot spot, we used a *Bgl*II-*Pvu*II restriction fragment derived from pSP2; this fragment contains sequences from the neighboring *BIK1* gene (Fig. 1). Chromatin in the *ARG4* region was examined using a *Bgl*II-*Eco*RV derived from pAK1; this fragment contains sequences from the 5′ end of *ARG4*. The *Bgl*II fragments representing the intact *HIS4* and *ARG4* regions were 3.0 and 5.0 kb, respectively. Quantification of the level of radioactivity in each band was done with a Molecular Dynamics PhosphorImager.

Since the DNase I digestion studies were done with chromatin isolated from meiotic cells, a meiosis-specific DSB in the *HIS4* region could generate a band of hybridization. The absence of such a band in chromatin samples that were not treated with DNase I indicates that the level of DSBs in *RAD50* cells is too low to represent a complicating factor.

Analysis of MNase-hypersensitive sites in chromatin. The chromatin preparation and MNase digestion procedures were adapted from the procedure of Bloom and Carbon (3). Cells (100 ml) in sporulation medium were collected at various time points in meiosis, harvested by centrifugation, and resuspended in TESM buffer (1.2 M sorbitol, 0.1 M EDTA, 0.2 M Tris-HCl [pH 9.1], 0.7% β-mercaptoethanol). The cells were incubated at room temperature for 10 min, harvested, and resuspended in SCE buffer (1 M sorbitol, 0.06 M EDTA, 0.1 M citric acid [pH 5.8]) plus 10 mg of Zymolyase 20 T. Cells were incubated at 30°C until spheroplast formation was complete (usually about 5 min). The spheroplasts were washed with SPC buffer [1 M sorbitol, 0.1 mM calcium chloride, 20 mM piperazine-*N*,*N*′-bis(ethanesulfonic acid) (PIPES; pH 6.3)] before being lysed with Ficoll solution (9% Ficoll 400, 0.5 mM calcium chloride, 20 mM PIPES [pH 6.3], 1 mM PMSF). The cell lysates were spun down at 20,000 × *g* for 20 min and then washed with SPC plus 1 mM PMSF. The pellets were resuspended in 1.2 ml of SPC plus 1 mM PMSF and incubated at 32°C for 3 min. MNase (Pharmacia) was then added at the concentration of 50 U/ml. Samples of 400 μl were collected at various times between 0 and 15 min after the addition of the nuclease. The control samples with naked DNA (10 μg) were digested under the same conditions except that the concentration of MNase was 500 U/ml. The reactions were terminated by addition of termination mix (50 μl of 10% SDS, 50 μl of 500 mM EDTA, 100 μl of 5 M NaCl). DNA was extracted and analyzed by the methods used for the DNase I experiments described above.

Relative DNase I sensitivities of *HIS4* and *ARG4* regions. For some experiments, we compared the DNase I sensitivities of the *HIS4* and *ARG4* regions. We first hybridized the filters to the *HIS4*-specific probe and quantitated the level of the intact (3.0-kb) *Bgl*II fragment at each enzyme concentration, using a Molecular Dynamics PhosphorImager. After the *HIS4* signal had decayed to low levels, we rehybridized the same filter to the *ARG4*-specific probe and quantitated the level of the intact (5.0-kb) *Bgl*II fragment for DNA samples from each enzyme concentration. For each strain, two independent experiments were performed.

The ratios of hybridization of the intact *ARG4* fragment to hybridization of the intact *HIS4* fragment were calculated for each enzyme concentration (0, 50, and 100 U). These ratios were normalized by dividing by the ratio of the intact *ARG4* fragment to the intact *HIS4* fragment at 0 U of enzyme. Thus, these normalized ratios have a value of 1 at 0 U of enzyme. A normalized ratio greater than 1 indicates that the *HIS4* chromatin is less sensitive to DNase I than the *ARG4* chromatin, and a value less than 1 indicates that the *HIS4* chromatin is more DNase I sensitive than the *ARG4* chromatin.

RESULTS

In this study, we examine chromatin structure in six strains with various levels of *HIS4* recombination hot spot activity (Table 1). These strains are isogenic except for changes introduced by transformation. DNY26 has the wild-type level of hot spot activity and wild-type promoter sequences (9, 16). PD15 has wild-type hot spot activity and a deletion of the TATAA sequence located upstream of *HIS4* (28); this deletion lowers the transcription rate but has no effect on hot spot activity. Strains QFY101 and MW154 have twofold-elevated levels of hot spot activity as a consequence of insertion of multiple Rap1p binding sites replacing the wild-type upstream sequences (9, 29, 30). QFY101 contains two Rap1p binding sites, and MW154 has a telomeric repeat with three such sites (9, 29). QFY102 is identical with QFY101 except for a mutation in one of the two Rap1p binding sites; this strain has about half the level of hot spot activity as the wild-type strain (9). QFY108 has no hot spot activity, as a consequence of a deletion of the Bas1p and Bas2p transcription factors (9). It should be emphasized that strains without hot spot activity completely lack

TABLE 1. Diploid strains

Strain(s)[a] (reference)	Relevant genotype	Hot spot activity[b]	Description of relevant alterations
QFY108 (9)	*his4-IR9* *bas1-1* *bas2-2* / *HIS4* *bas1-1* *bas2-2*	−	Deletion of transcription factors that bind to *HIS4* upstream region
QFY102 (9)	*his4-205* *his4-lopc* / *his4-205* *HIS4*	+/−	Replacement of normal upstream sequences with two Rap1p binding sites one mutant and one wild type)
DNY26 (16), [FX3] (9)	*his4-lop* / *HIS4*	+	None
PD15 (27)	*his4-445* *his4-Sal* / *his4-445* *HIS4*	+	Deletion of TATAA
QFY101 (9), [QFY106] (9)	*his4-204* *his4-lopc* / *his4-204* *HIS4*	++	Replacement of normal upstream sequences with two wild-type Rap1p binding sites
MW154 (28), [FX4] (9)	*his4-202* *his4-lopc* / *his4-202* *HIS4*	++	Replacement of normal upstream sequences with insertion of telomere sequences

[a] Strain names in brackets are isogenic *rad50S* derivatives.
[b] Assayed by measuring the percentage of total meiotic DNA with DSB at the *HIS4* locus (9). −, no hot spot, <0.2% DSB; +/−, weak hot spot, 1.7% DSB; +, wild-type hot spot, 3 to 5% DSB; ++, elevated hot spot activity, 16 to 19% DSB.

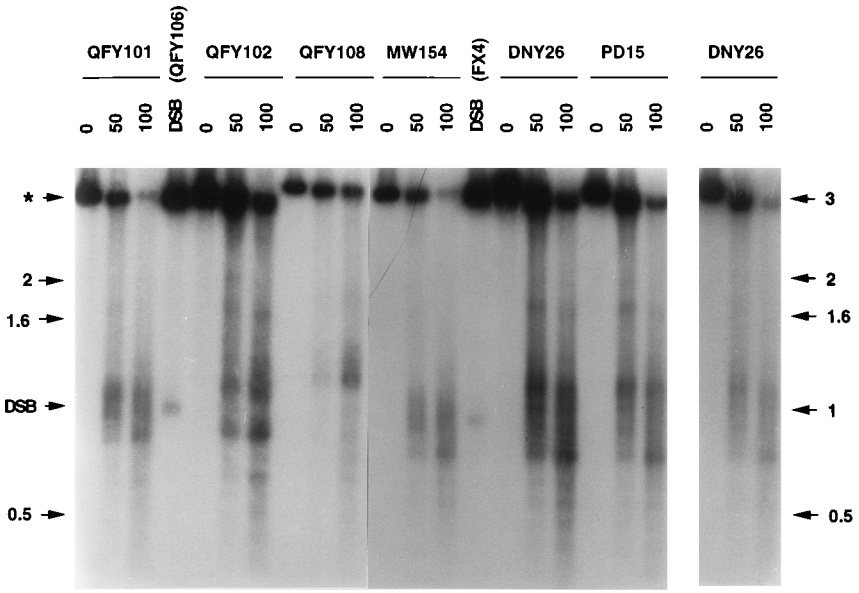

FIG. 2. DNase I-hypersensitive sites at the *HIS4* locus in meiotic chromatin derived from yeast strains with various levels of hot spot activity. Chromatin prepared from cells incubated for 6 h in sporulation medium was digested with 0, 50, 100 U of DNase I, as indicated above the lanes. DNA was extracted, treated with *Bgl*II, and examined by Southern analysis, using the hybridization probe depicted in Fig. 1. The lanes labeled DSB represent *Bgl*II-treated meiotic DNA samples isolated from *rad50S* derivatives of QFY101 (QFY106) and MW154 (FX4). The three rightmost lanes (marked DNY26) represent a lighter exposure of a portion of the adjacent filter. The arrow marked with an asterisk indicates the position of the 3-kb intact *HIS4 Bgl*II fragment. The numbers adjacent to other arrows represent the sizes (in kilobases) of DNA marker fragments.

hot spot-associated DSBs (9) and show no polarity of gene conversion (7) but still have a basal level of *HIS4* recombination (about one-third of the wild-type level).

Patterns of DNase I-hypersensitive sites at the *HIS4* meiotic recombination hot spot. We have previously observed that meiosis-specific DSBs appear about 4 to 6 h after cells are transferred into liquid sporulation medium (9). Thus, the chromatin isolated from cells after 6 h of sporulation is susceptible to in vivo cleavage by the endonuclease responsible for DSB formation. Consequently, our meiotic chromatin studies were performed on chromatin isolated from such cells.

Patterns of DNase I hypersensitivity of meiotic chromatin isolated from six strains with various levels of hot spot activity are shown in Fig. 2. A summary of the cleavage patterns is shown in Fig. 3. For all strains, the DNase I-hypersensitive sites occurred within the region upstream of *HIS4* and near the end of the neighboring *BIK1* gene. These hypersensitive sites represent a feature of the chromatin, since no similar sites were detected when naked DNA was treated with DNase I (Fig. 4a); in addition, meiotic chromatin derived from FX3 (*rad50S* derivative of DNY26; Table 1) had the same patterns of nuclease digestion with both DNase I and MNase as chromatin from DNY26 (data not shown).

In strain DNY26 (wild-type hot spot activity), the DNase I-hypersensitive sites covered an area of about 150 to 200 bp (Fig. 2, 3, and 4a). Four DNase I-sensitive sites can be seen. In strain QFY108 (no hot spot activity), in which the Bas1p and Bas2p transcription factors have been deleted, the number of hypersensitive sites is reduced to a single site mapping near the TATAA sequence.

Strains MW154 and QFY101 had elevated levels of hot spot activity (as discussed above) and similar patterns of DNase I-hypersensitive sites. In both strains, the numbers and positions of hypersensitive sites are different from those in the wild-type strain (Fig. 2). Strain QFY102, like QFY101, has two Rap1p binding sites replacing the normal *HIS4* upstream se-

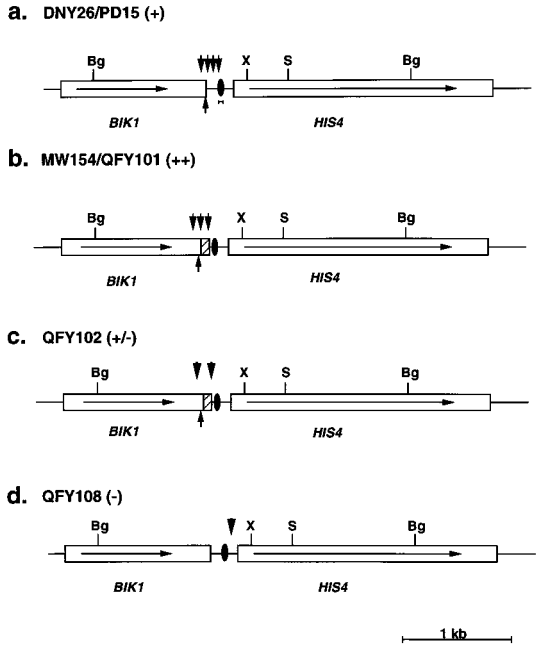

FIG. 3. Summary of DNase I cleavage patterns at the *HIS4* locus. As in Fig. 1, the DSB site is indicated by the small vertical arrow below the restriction map. Sites that are hypersensitive to DNase I are shown by large vertical arrows above the restriction map. Hot spot activities are indicated by + (wild-type activity), ++ (elevated activity), +/− (reduced activity), and − (no activity). (a) DNase I cleavage pattern of DNY26 (wild-type hot spot) and PD15 (promoter deletion). The extent of the deletion in PD15 is shown in brackets. (b) DNase I cleavage pattern of MW154 (telomere insertion in promoter) and QFY101 (double Rap1p binding site insertion). The cross-hatched rectangle shows the position of the oligonucleotide insertion that replaced the normal upstream sequences in the two strains. (c) DNase I cleavage pattern of QFY102 (one mutant and one functional Rap1p binding site). In this strain, the upstream region had two hypersensitive sites, but the region was not generally hypersensitive. (d) DNase I cleavage pattern of QFY108 (*bas1 bas2* double mutant). The hypersensitive region was contracted, and no strong hypersensitive sites were seen.

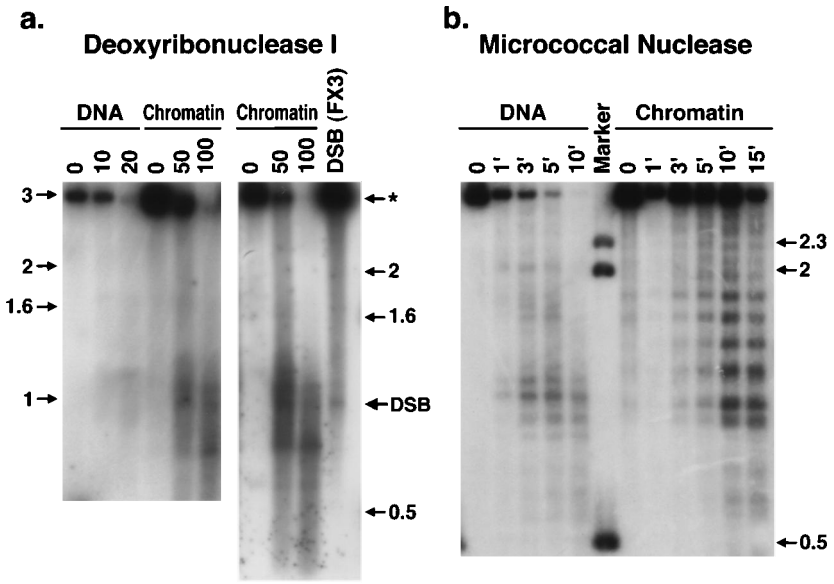

FIG. 4. DNase I and MNase cleavage patterns in naked DNA and chromatin derived from a strain (DNY26) with wild-type hot spot activity. The numbers adjacent to the arrows indicate the sizes of marker DNA fragments in kilobases. Procedures for DNA isolation and Southern analysis were the same as described for Fig. 2. (a) DNase I digestion patterns. The gel at the right shows the position of the meiosis-specific DSB compared with the DNase I pattern. The arrow marked with an asterisk shows the position of intact *Bgl*II restriction fragment. Numbers above the lanes represent units of DNase I. The lane marked DSB contains meiotic DNA from FX3, a *rad50S* derivative of DNY26. (b) MNase digestion patterns. Naked DNA or meiotic chromatin was treated with MNase as described in Materials and Methods. Numbers above the lanes represent times (in minutes) of treatment with MNase I. The same procedures for Southern analysis were used for both DNase I- and MNase-treated samples.

quences, but one of these binding sites is mutated, causing a reduction in hot spot activity. In this strain, there were two strong hypersensitive sites located at approximately the boundary positions of the hypersensitive sites of the wild-type strain (Fig. 2 and 3). One of these sites was located near the Rap1p binding site, and the other was located near the 3′ end of *BIK1*.

As described in the introduction, hot spot activity appears independent of the level of transcription at the *HIS4* (28) and *ARG4* (21) loci. The DNase I patterns for strains DNY26 (wild-type hot spot) and PD15 (TATAA deletion with wild-type hot spot activity) were very similar, indicating that the pattern of DNase I-hypersensitive sites is not substantially altered by reduction in the level of transcription or by absence of the TATAA-binding protein.

In summary, as expected from many studies of chromatin (reviewed in reference 10), the promoter region of *HIS4* is more sensitive to DNase I than the flanking coding sequences of *HIS4* and *BIK1*. The nuclease-sensitive site patterns are somewhat different in strains in which the transcription factors binding to the upstream region have been altered or in which protein-binding sites in the region have been altered.

Relative DNase I sensitivities of *HIS4* and *ARG4* hot spot regions. In addition to examining the pattern of DNase I-sensitive sites in chromatin, we measured the relative amounts of the intact *HIS4* and *ARG4* DNA fragments after treatment with different levels of DNase I in the six strains with different levels of hot spot activity. This comparison was made by consecutively hybridizing the same blot to *HIS4* and *ARG4* hybridization probes and measuring the amounts of intact *ARG4* and *HIS4* fragments. As described in detail in Materials and Methods, these data were normalized to yield a ratio of *ARG4*/*HIS4* DNase I sensitivity. A value less than 1 indicates that the *HIS4* locus is more sensitive to DNase I than the *ARG4* locus. As shown in Fig. 5, for most strains, the *HIS4* and *ARG4* loci were equally sensitive to DNase I at both concentrations of the enzyme. The *HIS4* region with the telomeric insertion (strain MW154) was somewhat more sensitive (relative to the *ARG4* locus) to DNase I (100 U), and the *HIS4* region from the strain with deletions of the Bas1p and Bas2p transcription factors (QFY108) was somewhat more resistant to DNase I treatment.

Since MW154 has the highest level and QFY108 has the lowest level of hot spot activity (9), there is a correlation between the relative DNase I sensitivity of the *HIS4* region and hot spot activity. This correlation is relatively weak, however,

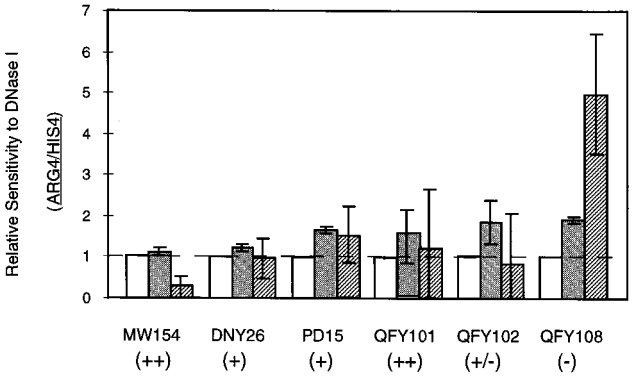

FIG. 5. Relative DNase I sensitivities of *HIS4* and *ARG4* meiotic chromatin. As described in Materials and Methods, we compared the DNase I sensitivities of *ARG4* and *HIS4* chromatin by measuring the relative amounts of digestion of the intact *ARG4* and *HIS4 Bgl*II fragments at various concentrations of DNase I. These values were normalized to give an *ARG4*/*HIS4* chromatin sensitivity ratio of 1 in samples that were not treated by DNase I. The data represent averages of two independent determinations. Open bars, undigested samples; stippled bars, 50 U of enzyme; striped bars, 100 U of enzyme. Brackets represent standard deviations. Hot spot activities are indicated as + (wild-type activity), ++ (elevated activity), +/− (reduced activity), and − (no activity).

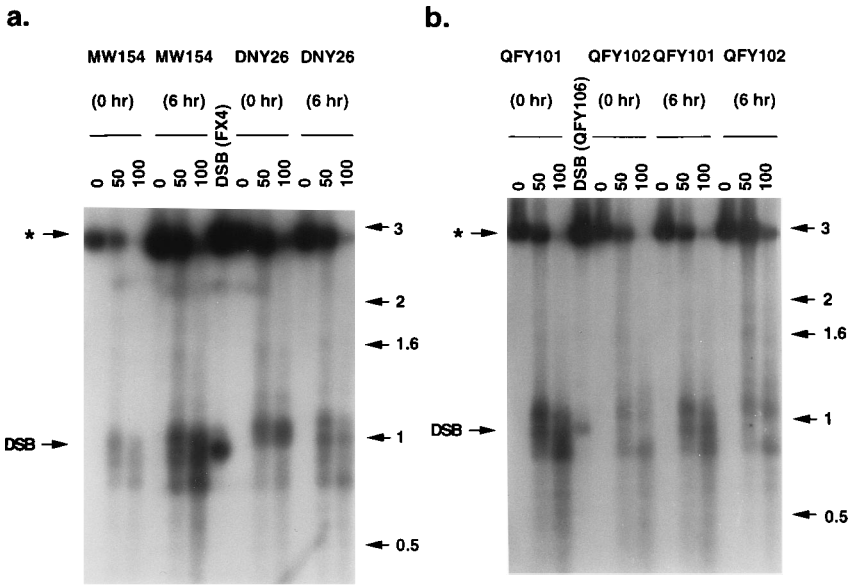

FIG. 6. Comparison of DNase I sensitivities of chromatin isolated from mitotic and meiotic cells. Chromatin preparations were made from mitotic cells or cells incubated for 6 h in sporulation medium. Chromatin was digested with DNase I and analyzed as described for Fig. 2. The arrow marked with an asterisk indicates the position of the intact *Bgl*II *HIS4* fragment. The numbers next to arrows show the sizes (in kilobases) of marker DNA fragments. The number of enzyme units used to treat each sample is indicated above each lane. (a) Comparison of patterns from MW154 (elevated hot spot activity) and DNY26 (wild-type hot spot). FX4 is a *rad50S* derivative of MW154. (b) Comparison of patterns from QFY101 (elevated hot spot activity) and QFY102 (reduced hot spot activity). QFY106 is a *rad50S* derivative of QFY101.

since QFY101 had almost as much hot spot activity as MW154 (9) but showed no increased sensitivity to DNase I.

Relationship between DNase I-hypersensitive sites and the position of the DSB. Wu and Lichten (31) reported a striking correspondence between the positions of DNase I-hypersensitive sites and meiosis-specific DSBs. In our study, the sites for DSB formation appear to represent a subset of the DNase I cleavage sites. Since DNase I-hypersensitive sites and DSB sites have not been mapped with single base pair resolution, this conclusion is tentative. As shown in Fig. 1 to 3, the bands representing DSBs in *rad50S* derivatives of MW154, QFY101, and DNY26 have a narrower distribution than the region of hypersensitive sites in these strains. Both MW154 and QFY101 have three bands of hybridization in the hypersensitive region, and the position of the DSB is close to the middle of these bands. These results indicate that a DNase I-hypersensitive site may be necessary but is not sufficient for a site for DSB formation. The results also do not rule out the possibility of hot spots for DSB formation that are not DNase I sensitive.

Chromatin structure at the *HIS4* recombination hot spot in mitotic and meiotic cells. In a previous study, Wu and Lichten (31) reported that chromatin isolated from mitotic and meiotic cells had approximately the same patterns of DNase I hypersensitivity. In contrast, Ohta et al. (18) reported that an MNase-hypersensitive site correlating with the position of a DSB was about threefold more nuclease sensitive in chromatin isolated from meiotic cells. To determine whether meiosis-specific alterations occurred at the *HIS4* hot spot, we examined chromatin isolated from cells prior to incubation in sporulation medium and from cells incubated for 6 h in sporulation medium. DNase I sensitivity was examined for strains MW154, DNY26, QFY101, and QFY102; in addition, chromatin isolated from strains MW154 and DNY26 was examined with MNase.

In the strain with wild-type hot spot activity (DNY26), the DNase I-hypersensitive region was expanded in meiotic cells relative to the size of this region in mitotic cells. The patterns of DNase I sensitivity of meiotic and mitotic chromatin, however, were the same for strains MW154, QFY101, and QFY102 (Fig. 6). The pattern of mitotic hypersensitive sites in DNY26 is similar to the meiotic pattern observed for QFY108 (Fig. 2 and 3). Since QFY108 lacks Bas1p and Bas2p, one interpretation of this result is that the synthesis (or binding) of these proteins is induced in meiosis.

We also examined meiotic and mitotic chromatin treated with MNase. As shown in Fig. 4b and 7, ladders of bands, separated by 150 to 200 bp, were observed for strains MW154 and DNY26. Although naked DNA is not cleaved randomly by MNase, the pattern of cleavages observed in chromatin is different from that observed for naked DNA (Fig. 4b). Ladders of bands after MNase treatment often reflect strongly phased nucleosomes (10). Although the ladders were somewhat different between the two strains, the meiotic and mitotic patterns for the two strains were similar. This result indicates that none of the MNase-sensitive sites near the *HIS4* hot spot region are preferentially activated during meiosis, in contrast to the results obtained at *ARG4* (18). In addition, the position of the DSB in FX4 (the *rad50S* derivative of MW154) did not correlate with any of the sites that were very sensitive to MNase in MW154 (Fig. 7).

DISCUSSION

One simple model to explain meiotic recombination hot spots is that these such regions represent open chromatin, allowing DNA access to meiosis-specific endonucleases. If DNase I and MNase represent good probes for the openness of chromatin, our analysis of the *HIS4* hot spot makes this simple model unlikely. It is clear that the DNase I- and MNase-sensitive regions are larger than the region in which the DSB occurs. At the level of resolution of the Southern blot, the DSB, in at least some strains, appears to be at the approximate

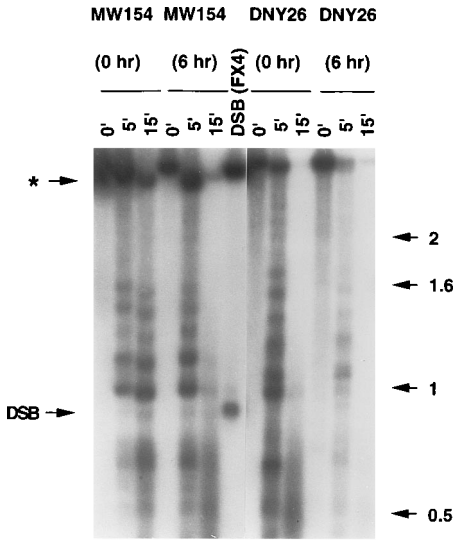

FIG. 7. Patterns of MNase sensitivity in chromatin isolated from mitotic and meiotic cells. Chromatin was isolated from mitotic cells or cells incubated for 6 h in sporulation medium and digested with MNase for various times (in minutes; see Materials and Methods). DNA was extracted from the MNase-treated chromatin, treated with *Bgl*II, and examined by Southern analysis. The DNA sample for detection of the DSB was prepared as described previously (9). The arrow with an asterisk shows the position of the intact *HIS4 Bgl*II fragment, and numbers next to other arrows represent the sizes (in kilobases) of marker DNA fragments.

position of one of the DNase I-hypersensitive sites. DNA sequence analysis of the position of the DSB and the hypersensitive site would be necessary to confirm this conclusion. The position of the DSB does not correlate with any of the strong MNase-hypersensitive sites.

Wu and Lichten (31) previously showed a correlation between DNase I-hypersensitive regions and the positions of DSBs. In general, these experiments were done with large DNA fragments, preventing an analysis of hypersensitive sites within the hypersensitive region. In subsequent analyses, however, these workers found that the patterns of DSB formation and nuclease-hypersensitive sites were not always identical (13, 32). Ohta et al. (18) reported a correlation between the position of the DSB and a meiosis-enhanced MNase-hypersensitive site. A difference between our study and that of Ohta et al. (18) is that the *HIS4* locus has phased nucleosomes and the *ARG4* locus does not. The strongly MNase-hypersensitive sites produced by the phasing may prevent detection of weak MNase-sensitive sites associated with DSB formation. Alternatively, phased nucleosomes flanking the hot spot region may interfere with MNase cleavage but not DNase I cleavage.

Our data, as well as data from other laboratories discussed above, are consistent with three possibilities. First, an open chromatin structure is sufficient for creation of a meiotic chromatin hot spot, but neither DNase I nor MNase is a perfect probe for this openness. Second, a hot spot may require open chromatin and an additional factor (for example, interaction with a DNA-bound protein near the hot spot). Third, a hot spot may require not open chromatin but only a factor (such as interaction with a transcription factor) that is often associated with open chromatin.

Although none of these models can be excluded by the data, our preferred model is that efficient production of a DSB requires an interaction between the meiosis-specific endonuclease and transcription factors. Since we observe a strong hot spot in QFY101, in which two Rap1p binding sites replace the normal arrangement of transcription factor binding sites, the Rap1p may represent the relevant interacting transcription factor. It is also possible that the interaction between transcription factors and the meiotic endonuclease is less direct. It has been suggested that Rad50p, Mre11p, and Xrs2p may form a complex that binds to open chromatin and interacts with the meiosis-specific endonuclease (11, 20). This complex may act as a bridge between the meiotic endonuclease and Rap1p or other transcription factors. In this model, transcription factors act to anchor the endonuclease to the chromosome in order to cleave the chromosome efficiently. Thus, the levels of DSBs are determined by the affinity of the endonuclease for the DNA substrate via the transcription factor bridge. Since the levels of hot spot activity differ in different upstream regions, we suggest that some transcription factors are more efficient at recruiting the meiotic endonuclease than other transcription factors. Alternatively, different transcription factors may recruit the endonuclease with similar efficiencies, but the degree of DNA accessibility shows locus-to-locus variation. A similar model has been proposed to explain the function of transcription activation domains (5). These workers suggest that the function of these domains is to stabilize the interaction of the TATAA-binding element to its binding site.

Since promoter regions are usually devoid of nucleosomes, these regions are often sensitive to DNase I (10). We find that a deletion of the *HIS4* TATAA region that substantially reduces transcription has no obvious effect on sensitivity of this region to DNase I. In other studies of *S. cerevisiae* (12, 14), it was also shown that DNase I hypersensitivity of the region does not necessarily correlate with the level of transcription. Strain QFY108, which had deletions of the Bas1p and Bas2p transcription factors, had a contracted hypersensitive region, indicating that the binding of these factors is required for wild-type levels of DNase I sensitivity of the *HIS4* promoter. In addition, Devlin et al. (8) reported that the binding of Rap1 to the *HIS4* promoter increased the sensitivity of the region to MNase. These results taken together show that nuclease-sensitive sites in the *HIS4* promoter are correlated with the binding of the Bas1p, Bas2p, and Rap1p transcription factors but not with the TATAA-binding protein.

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