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Genetics

Transcription factors are required for the meiotic recombination hotspot at the *HIS4* locus in *Saccharomyces cerevisiae*

(heteroduplex/chromatin/telomere)

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ABSTRACT **The full activity of a recombination initiation site located 5′ of *HIS4* requires the binding of the transcription factors RAP1, BAS1, and BAS2. Two RAP1 binding sites can substitute for the wild-type initiation site. A 51-bp region of telomeric DNA inserted upstream of either *HIS4* or *ARG4* very strongly stimulates recombination. We suggest that the ability of transcription factors to induce recombination is a consequence of an altered chromatin structure that favors the entry of proteins that initiate recombination, rather than an effect of these factors on transcription.**

During meiosis in the yeast *Saccharomyces cerevisiae*, three types of recombination can be monitored: crossovers, gene conversions, and postmeiotic segregations. In a heterozygous diploid (alleles *A* and *a*), tetrads with the aberrant segregation patterns 3*A*:1*a* or 1*A*:3*a* spores represent gene conversions; spore colonies that have *A*/*a* sectors represent postmeiotic segregation events. Most gene conversions reflect heteroduplex formation between different alleles, followed by repair of the resulting mismatch, whereas postmeiotic segregation events reflect heteroduplexes in which the mismatch is not repaired (1). Since conversion and postmeiotic segregation are associated with crossovers, heteroduplexes are likely to be an intermediate in all recombination events. Below, we will describe aberrant segregation events, using the nomenclature derived from eight-spored fungi [conversions indicated by 6*A*:2*a* or 2*A*:6*a* segregation, and postmeiotic-segregation tetrads as 5*A*:3*a* (two *A* spore colonies, one *a* spore colony, and one sectored colony) or 3*A*:5*a*].

Some regions of chromosomes have unusually high levels of aberrant meiotic segregation (1). At the *ARG4* and *HIS4* loci, these hotspots map upstream of the 5′ end of the gene (2–5). Deletions of these sequences lower the frequency of aberrant segregation for closely linked markers. If the hotspot deletion is heterozygous, a disparity in the classes of aberrant segregation for closely linked markers is observed in the direction expected if the chromosome with the hotspot receives information from the chromosome without the hotspot (1). One interpretation of this observation is that the chromosome with the hotspot contains a DNA lesion (either a double-strand break or a single-strand gap) that initiates the exchange; in several recombination models, the chromosome with the lesion is expected to be the recipient of information (1). At *ARG4*, the hotspot colocalizes with the site of a double-strand break (2, 3).

One *HIS4* deletion that reduces hotspot activity contains binding sites for the transcription factors GCN4, RAP1, BAS1, and BAS2 (6–8). The binding of RAP1 to the region upstream of *HIS4* is necessary for full hotspot activity (4); however, this effect of this binding is not a direct consequence of an induction of transcription (9). We report here that the binding of BAS1 and BAS2 is necessary in conjunction with the binding of RAP1 for the *HIS4* hotspot to function.

MATERIALS AND METHODS

Plasmids. The plasmid pMW31 (9) contained a mutated copy of *GCN4* (650-bp deletion of the region encoding the DNA binding site) with a *URA3* gene inserted into the remaining coding sequence. Plasmids AB328 and AB289 (provided by K. Arndt, Cold Spring Harbor Laboratory) contained mutant derivatives of *BAS2* (*bas2-2*) and *BAS1* (*bas1-1*), respectively, inserted into YIp5.

Plasmid pMW43 had a palindromic oligonucleotide (5′-TAGTACTGTATGTACATACAGTAC-3′) inserted into the *Ase* I site of B189 [provided by B. Ruskin and G. Fink, Massachusetts Institute of Technology; *Sau*3A1 fragment with *HIS4* upstream sequences inserted into the *Bam*HI site of B142 (a YIp5 derivative lacking the *Pvu* II site)]. The resulting mutation is *his4-201*, an insertion in the BAS2 binding site between bp −222 and −223 (Fig. 1). Plasmid pPD5 contained a *Sau*3A1 fragment with the *his4-Δ52* mutation (5, 10) and a *Sau*3A1 fragment derived from pBR322 inserted into the *Bam*HI site of B142. Plasmid pDN22 had an *Xho* I–*Bgl* II fragment containing *his4-IR9* inserted into *Sal* I/*Bam*HI-treated YIp5 (11). Plasmid pDN13 contained the allele *his4-lopc* on an *Xho* I–*Bgl* II fragment inserted into YIp5 (12).

Derivatives of pPD5 were pMW50 [annealed oligonucleotides 1680 and 1681 (Fig. 2*c*) inserted into the *Xho* I site in the orientation 5′-1680-3′ to produce *his4-202*], pMW55 [annealed oligonucleotides 5416 and 5417 (Fig. 2*a*) inserted into the *Xho* I site in the orientation 5′-5417-3′ to produce *his4-204*], and pMW56 [annealed oligonucleotides 5422 and 5423 (Fig. 2*b*) inserted into the *Xho* I site in the orientation 5′-5423-3′ to produce *his4-205*].

Plasmid pMW52 had a *Bam*HI–*Bgl* II fragment of *ARG4* inserted into the *Bam*HI site of the YIp5 derivative B142. pMW53 was derived by inserting the annealed oligonucleotides 1680 and 1681 (after treatment with DNA polymerase to make blunt ends) into the *Hpa* I site (position −319 relative to the *ARG4* initiation codon) of pMW52; the insertion was in the orientation 5′-1681-3′ and created the Arg$^+$ allele *arg4-tel*.

Yeast Strains. All haploid yeast strains were derived by transformation from either AS4 (*MATα trp1 arg4 tyr7 ade6 ura3*) or AS13 (*MAT***a** *leu2 ade6 ura3*) by one-step or two-step transplacement procedures (14, 15). Below, we indicate the haploid strain, followed by the name of the parental strain from which the haploid was derived, the plasmid used in the construction, the procedure used [one-step (OS) or two-step (TS) transplacement], and the name of the mutation introduced by the transformation: DNY47 (AS13, pDN22, TS,

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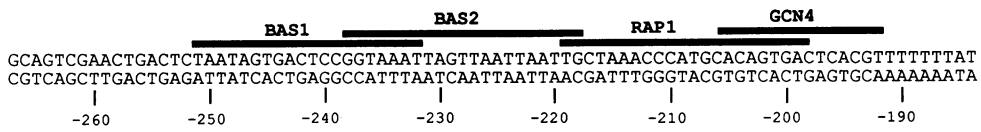

FIG. 1. Binding sites for transcription factors located upstream of *HIS4*. Numbers indicate positions relative to the initiation codon of *HIS4*. Horizontal bars represent protected regions of DNA (based on figure 4c of ref. 7 and figure 2 of ref. 8). The *his4-Δ52* deletion extends from −316 to −144.

his4-IR9); MW77 (DNY47, AB289, TS, *bas1-1*); MW78 (AS4, AB289, TS, *bas1-1*); MW71 (AS4, AB328, TS, *bas2-2*); MW62 (AS13, AB328, TS, *bas2-2*); MW67 (MW62, pDN22, TS, *his4-IR9*); MW9 (AS4, pMW31, OS, *gcn4*); MW43 (AS13, pMW43, TS, *his4-201*); MW42 (MW43, pMW31, OS, *gcn4*); MW48 (MW42, pDN22, TS, *his4-IR9*); PD63 (AS4, pPD5, TS, *his4-Δ52*); MW82 (PD63, pMW55, TS, *his4-204*); MW83 (PD63, pMW56, TS, *his4-205*); PD57 (AS13, pPD5, TS, *his4-Δ52*); MW68 (PD57, pMW50, TS, *his4-202*); MW73 (PD63, pMW50, TS, *his4-202*); MW72 (MW68, pDN13, TS, *his4-lopc*); MW79 (AS13, pMW53, TS, *arg4-tel*); MW81 (AS4, pMW53, TS, *arg4-tel*). The strain MW17 was a *ura3* derivative of MW9 isolated as a spontaneous mutant resistant to 5-fluoroorotate.

Diploid yeast strains were constructed by mating the appropriate haploids: MW134 (MW42 × MW9), MW138 (MW48 × MW17), MW150 (MW68 × PD63), MW153 (MW71 × MW67), MW154 (MW73 × MW72), MW158 (MW77 × MW78), MW159 (MW79 × AS4), MW160 (MW81 × MW79), MW162 (MW82 × PD57), MW163 (MW73 × PD57), and MW164 (MW83 × PD57).

Media and Genetic Techniques. Standard methods were used for sporulation and tetrad analysis (16), except that cells were sporulated at 18°C, a temperature that results in high levels of aberrant segregation at the *HIS4* locus in our genetic background (12). Standard allelism and complementation tests were performed to score heterozygous *his4* mutations.

RESULTS

Deletion analysis indicates that a 172-bp region located between the 5′ end of *HIS4* and the 3′ end of *BIK1* stimulates recombination for both genes (5). Four proteins (GCN4, RAP1, BAS1, and BAS2) have been shown to bind in this region (Fig. 1) to activate *HIS4* transcription (6–8). We showed previously that the binding of RAP1 (4), but not GCN4 (9), is required for hotspot activity. To examine the effects of the other transcription factors, we did several types of experiments. First, we examined the effects of *bas1* and *bas2* mutations on the frequency of aberrant segregation of a *HIS4* marker. Second, we examined the effects of mutating the BAS2 binding site upstream of *HIS4*. Third, we constructed strains with homozygous deletions of the wild-type hotspot and a heterozygous insertion (at the same position as the original hotspot) of oligonucleotides containing various protein-binding sequences; to determine whether this insertion had hotspot activity, we examined both the frequency of aberrant segregation of the heterozygous insertion and the disparity in the types of segregation events. Fourth, we examined the effects of telomeric insertions on the frequency of aberrant segregation of markers within *HIS4* or *ARG4*.

Requirement of BAS1 and BAS2 for Hotspot Activity. To determine whether the BAS1 protein was necessary for the hotspot, we constructed a diploid strain (MW158) that was homozygous for a deletion of the *BAS1* gene and heterozygous for a mutation (*his4-IR9*) within the coding sequences of *HIS4*. Although the *bas1* mutation reduces *HIS4* gene expression (6), haploid strains with the *bas1 HIS4* genotype are His$^+$. Since *his4-IR9* is a palindromic insertion, which leads to poorly repaired mismatches (12), most of the aberrant segregations observed for this allele were postmeiotic segregation events rather than gene conversions.

The frequency of aberrant segregation in MW158 was 15% (Table 1). This frequency is significantly less than that observed for the same mutant allele in an isogenic strain with the wild-type *BAS1* gene (37% in DNY48, Table 1). The decrease is similar to that observed in strains homozygous for a mutant RAP1 binding site and heterozygous for a palindromic insertion at the same position as *his4-IR9* (18%; ref. 4). In addition, the recombination distance between *HIS4* and *LEU2* was reduced in MW158 compared with a wild-type strain (Table 2). The effect of the *bas1* mutation does not represent a global reduction of recombination, since the aberrant segregation frequencies of other heterozygous markers in MW158 were unaffected (data not shown). The simplest interpretation of these results is that the function of the hotspot requires BAS1.

We also examined recombination in a strain (MW153) that was homozygous for a deletion of *BAS2* and heterozygous for *his4-IR9*. Haploid strains of the *bas2 HIS4* genotype are His$^+$ (6). The frequency of aberrant segregation was 17% (Table 1), significantly ($\chi^2 = 27$; $P < 0.001$) less than that observed for the same mutant allele in the isogenic wild-type strain DNY48 (37%). Crossovers were also reduced (Table 2). The decrease

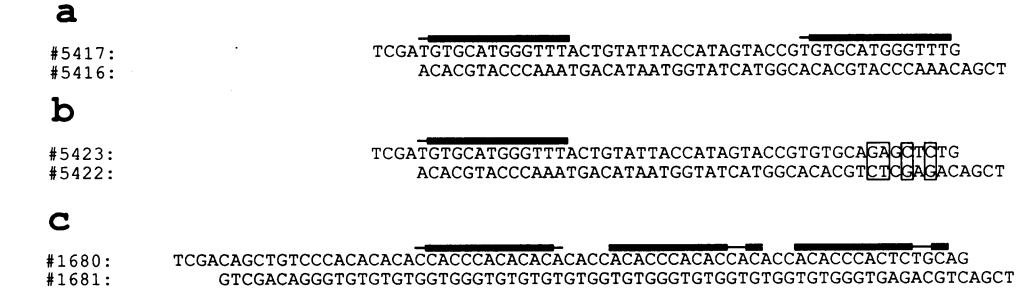

FIG. 2. Oligonucleotide insertions containing protein-binding sites. Pairs of complementary oligonucleotides are shown in their annealed configuration. The top strand is shown 5′ to 3′ from left to right. Numbers at left represent the individual oligonucleotides described in *Materials and Methods*. Bars above the sequence represent potential RAP1 binding sites, with thick lines over bases with an exact match to the consensus sequence (13) and thin lines over the bases that differ from the consensus sequence. (*a*) Oligonucleotides (used to construct *his4-204*) containing a duplication of the RAP1 binding site normally found in one copy upstream of *HIS4*. (*b*) Oligonucleotides (used to construct *his4-205*) containing one mutant and one wild-type RAP1 binding site. The bases that were changed to mutate the binding site are boxed. (*c*) Oligonucleotides (used to construct *his4-202* and *arg4-tel*) containing telomeric sequences.

Table 1. Number of tetrads with aberrant (Ab) segregation patterns for *HIS4* markers in strains with mutations that affect the binding of *HIS4* transcription factors

Strain	Relevant mutation(s)*	Segregation 6:2	2:6	8:0	0:8	5:3	3:5	Ab. 4:4	Other PMS	Total tetrads	% Ab.
MW158	$\frac{bas1\text{-}1\ his4\text{-}IR9}{bas1\text{-}1\ HIS4}$	7	6	0	0	16	15	5	1	335	15
DNY48†	$\frac{his4\text{-}IR9}{HIS4}$	15	15	0	1	50	44	8	6	379	37
MW153	$\frac{bas2\text{-}2\ his4\text{-}IR9}{bas2\text{-}2\ HIS4}$	3	5	0	0	14	14	4	2	247	17
MW134	$\frac{his4\text{-}201}{HIS4}$	10	21	0	1	25	50	8	7	290	42
MW138‡	$\frac{his4\text{-}201}{HIS4}$	2	30	0	0	16	52	5	8	299	38
	$\frac{his4\text{-}IR9}{HIS4}$	5	11	0	0	19	35	7	4		27
MW162	$\frac{his4\text{-}\Delta52}{his4\text{-}204}$	29	58	2	7	1	3	0	1	355	28
MW164	$\frac{his4\text{-}\Delta52}{his4\text{-}205}$	29	37	0	1	3	4	0	0	431	17
MW150	$\frac{his4\text{-}202}{his4\text{-}\Delta52}$	14	68	0	24	2	3	0	5	247	47
MW163	$\frac{his4\text{-}\Delta52}{his4\text{-}202}$	11	74	0	33	3	8	0	5	314	43
MW154	$\frac{his4\text{-}202\ his4\text{-}lopc}{his4\text{-}202\ HIS4}$	17	9	1	1	56	72	20	55	321	72
DNY26§	$\frac{his4\text{-}lopc}{HIS4}$	18	15	1	0	40	37	8	2	394	31

Tetrads with 6:2 and 2:6 segregation reflect single conversion events; 5:3 and 3:5 tetrads reflect single postmeiotic segregation (PMS) events; 8:0 and 0:8 reflect double conversion events (12); aberrant 4:4 segregation reflects tetrads with one spore colony of each parental genotype and two PMS spore colonies. The "Other PMS" class reflects tetrads with one conversion event and one PMS event (7:1 or 1:7) or tetrads with more than one PMS event that are not aberrant 4:4 tetrads.
*The genotypes written above and below the lines signify markers contributed by the AS13- and AS4-derived parental strains, respectively.
†Described previously (11).
‡This strain was heterozygous for two markers; aberrant segregation for each marker is shown separately.
§Described previously (12).

in recombination was not seen for other heterozygous markers in the cross (data not shown).

Although the simplest interpretation of these experiments is that the binding of BAS1, BAS2, and RAP1 are required for hotspot activity, we cannot rule out the possibility that BAS1 and BAS2 affect *HIS4* recombination indirectly. To exclude this possibility for BAS2, we examined the effects of a mutation within the BAS2 binding site upstream of *HIS4* on meiotic recombination in strains (MW134 and MW138) with wild-type levels of BAS1 and BAS2. Each of these strains was heterozygous for a palindromic insertion within the BAS2 binding site at the *Ase* I restriction site (−225 to −220 in Fig. 1); the resulting mutation was denoted *his4-201*. To score *his4-201* genetically, we made MW134 and MW138 homozygous for *gcn4*, since Arndt *et al.* (6) had showed that strains lacking *gcn4* and *bas2* were His$^-$, although strains with mutations in either *GCN4* or *BAS2* were His$^+$. Similarly, we found that strains with a *gcn4* mutation or a mutation of the *BAS2* binding site were His$^+$, whereas the strains with both alterations had a leaky His$^-$ phenotype. This result strongly indicates that the insertion in *his4-201* prevents binding of BAS2.

Table 2. Number of tetrads with parental ditype (PD), nonparental ditype (NPD), or tetratype (T) segregation patterns for the heterozygous *HIS4* and *LEU2* markers in strains with mutations that affect the binding of *HIS4* transcription factors

Strain	Relevant genotype	Segregation pattern (*LEU2–HIS4*) PD	NPD	T	Map distance, centimorgans
DNY48	$\frac{his4\text{-}IR9}{HIS4}$	182	10	133	30
MW153	$\frac{bas2\text{-}2\ his4\text{-}IR9}{bas2\text{-}2\ HIS4}$	144	4	70	22
MW158	$\frac{bas1\text{-}1\ his4\text{-}IR9}{bas1\text{-}1\ HIS4}$	185	4	110	22
PD81*	$\frac{his4\text{-}\Delta52\ his4\text{-}lopc}{his4\text{-}\Delta52\ HIS4}$	184	4	79	19
MW154	$\frac{his4\text{-}202\ his4\text{-}lopc}{his4\text{-}202\ HIS4}$	75	23	133	59

Map distances in fungi can be calculated by determining the relative frequencies of PD, NPD, and T tetrads involving two heterozygous linked markers (17).
*Construction was described previously (5).

The aberrant segregation frequency of the *his4-201* allele in MW134 was 42% (Table 1). This frequency, although high, is less than that observed for mutations in the initiating codon of *HIS4* in strains with wild-type upstream sequences (48%; ref. 5). In addition, unlike other palindromic insertions examined previously (11, 12), *his4-201* showed a significant ($\chi^2 = 12$; $P < 0.001$) excess of aberrant segregation events in which the *HIS4* allele received information from the *his4-201* allele (i.e., 3:5 and 2:6 events exceeded 5:3 and 6:2 events). Since previous results with the *HIS4* and other hotspots (1) indicate that the chromosome with the hotspot functions as a recipient during recombination, this result suggests that the insertion in the BAS2 binding site reduces hotspot activity.

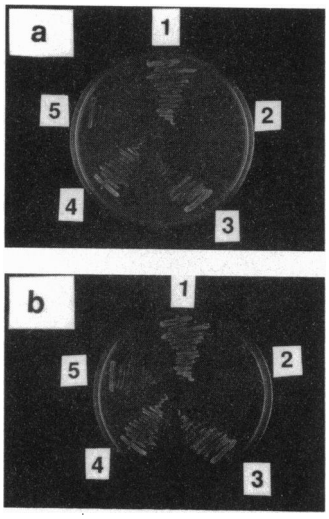

FIG. 3. Expression of *HIS4* with different upstream sequences. Haploid yeast strains with modifications of the region upstream of *HIS4* (otherwise isogenic) were streaked on a plate containing medium lacking histidine. The plate was incubated at 30°C and photographed after 3 days (*a*) or 5 days (*b*). Code: 1, AS4 (wild-type upstream sequences); 2, PD63 (*his4-Δ52*; deletion of binding sites for BAS1, BAS2, RAP1, and GCN4); 3, MW82 (*his4-204*; two RAP1 binding sites replacing normal upstream sequences); 4, MW73 (*his4-202*; telomeric DNA replacing normal upstream sequences); 5, MW83 (*his4-205*; one mutant and one wild-type RAP1 binding site replacing normal upstream sequences). Strains 1, 3, and 4 are His^+, strain 2 is His^-, and strain 5 has a leaky His^- phenotype.

The strain MW138 is heterozygous for both *his4-201* and *his4-IR9*, and segregation frequencies for both alleles can be monitored separately by complementation tests. The *his4-201* mutation had the same pattern of conversion disparity as was observed in MW134. The aberrant segregation frequency for *his4-IR9* was 27% (Table 1), a significant ($\chi^2 = 6.6$; $p = 0.01$) reduction from the 37% frequency observed for *his4-IR9* in a strain without *his4-201*. In addition, there was a disparity in the classes of aberrant segregation of the *his4-IR9* mutation, indicating that the chromosome with the *his4-201* mutation functions less efficiently as a recipient of information than the wild-type chromosome; this disparity was not found in a strain with *his4-IR9* and wild-type upstream sequences (Table 1).

An Insertion with Two RAP1 Binding Sites, but No Binding Sites for BAS1 or BAS2, Stimulates Recombination. To determine whether other configurations of DNA-binding proteins could create a recombination hotspot, we examined the effects of replacing the normal upstream region of *HIS4* with an oligonucleotide that had two RAP1 binding sites, but no sites for binding of BAS1 or BAS2 (Fig. 2*a*). In the strain MW162, the region binding the transcriptional activators was deleted from both chromosomes (*his4-Δ52*) and, on one of the chromosomes, the oligonucleotide with two RAP1 sites was substituted at the endpoints of the deletion (*his4-204*); haploid strains with *his4-Δ52* were His^- (ref. 10 and Fig. 3), whereas strains with the *his4-204* mutation were His^+ (Fig. 3). The level of recombination observed for the heterozygous insertion (28% for *his4-204*) was higher than that observed for the heterozygous wild-type hotspot previously (17%, *his4-Δ52/HIS4*, PD58; ref. 5), although this comparison may be affected by the different sizes of the heterologies involved (51 bp for the oligonucleotide and 172 bp for the *his4-Δ52* deletion). The heterozygous insertion had conversion disparity in the direction that would be expected if the oligonucleotide had hotspot activity. We also examined a strain (MW164) that was identical to MW162 except that the oligonucleotide inserted upstream of *HIS4* had one wild-type and one mutant RAP1 binding site (Fig. 2*b*). This insertion (*his4-205*), which resulted in a leaky His^+ phenotype (Fig. 3), had a lower level of aberrant segregation than *his4-204* (17% instead of 28%), and no significant disparity was observed in the classes of segregants. These results indicate that two (but not one) RAP1 binding sites can create a meiotic recombination hotspot, in the absence of BAS1 and BAS2 binding sites.

Stimulation of Recombination by Telomeric Sequences. Telomeric sequences in yeast can be abbreviated poly($dG_{1-3}dT$) and bind several proteins (including RAP1) (13, 18). We constructed strains (MW150 and MW163) that were homozygous for the *his4-Δ52* deletion (removing the wild-type hotspot) and heterozygous for the telomere insertion (*his4-202*). This insertion (Fig. 2*c*), which resulted in a His^+ phenotype (Fig. 3), had a high frequency of aberrant segregation (43–47%) and a strong disparity in the classes of aberrant segregants, indicating that the telomeric sequences created a recombination hotspot.

In a strain (MW154) that was homozygous for the telomeric insertion and heterozygous for the *his4-lopc* mutation, we found an aberrant segregation frequency of 72%, the highest reported for any marker in any yeast strain. In an isogenic strain with the wild-type hotspot (DNY26), the frequency of aberrant segregation of *his4-lopc* is 31% (12). The telomeric insertion also strongly stimulated crossovers (Table 2).

To determine whether this insertion could function in more than one context, we constructed two related strains in which a mutation in the *ARG4* coding sequence (*arg4-17*) was heterozygous and the telomeric insertion (located about 300 bp upstream of the initiating codon) was either heterozygous (MW159) or homozygous (MW160). The frequency of aberrant segregation of *arg4-17* without any insertion was 9% in our genetic background (Table 3) and was elevated to 28% in MW159 and to 52% in MW160.

Effects of Protein-Binding Sequences on *HIS4* Gene Expression. A deletion (*his4-Δ52*) that removes the binding sites for

Table 3. Number of tetrads with aberrant (Ab) segregation patterns at *ARG4* in strains with wild-type upstream sequences and in strains with telomeric insertions

Strain	Relevant mutation(s)	Segregation									
		6:2	2:6	8:0	0:8	5:3	3:5	Ab. 4:4	Other PMS	Total tetrads	% Ab.
Composite*	$\frac{ARG4}{arg4\text{-}17}$	124	110	4	5	0	0	0	0	2575	9
MW159	$\frac{arg4\text{-}tel}{} \frac{ARG4}{arg4\text{-}17}$	27	41	0	6	0	0	0	0	358	21
MW160	$\frac{arg4\text{-}tel}{arg4\text{-}tel} \frac{ARG4}{arg4\text{-}17}$	74	67	7	2	1	4	0	1	300	52

The designations for aberrant segregation classes are described in the legend to Table 1.
*Composite of data from strains with isogenic AS4 and AS13 genetic backgrounds (total of 2575 tetrads) that have wild-type sequences upstream of *ARG4*.

BAS1, BAS2, GCN4, and RAP1 results in a His^- phenotype (10). Strains that are singly mutant for BAS1, BAS2, or GCN4 or have a mutant RAP1 binding site are His^+ with somewhat diminished *HIS4* expression (6, 8). Strains with mutations in both *BAS1* (or *BAS2*) and *GCN4* have a leaky His^- phenotype (6). We found that strains containing an insertion with two RAP1 binding sites or a telomeric insertion substituting for the normal upstream region were His^+; strains with insertions containing a single RAP1 binding site substituting for the normal upstream region had a leaky His^- phenotype (Fig. 3). Thus, for both gene expression and recombination hotspot activity, novel arrangements of DNA-binding sequences can sometimes effectively substitute for the wild-type arrangement.

DISCUSSION

There are three plausible mechanisms to explain why the binding of transcription factors stimulates recombination. (*i*) Transcription may lead directly to high levels of meiotic recombination. Since the level of *HIS4* meiotic recombination is unaffected by a *HIS4* promoter deletion that reduces transcription more strongly than mutations of *BAS1* or *BAS2* (9), we do not favor this possibility. (*ii*) There may be a specific interaction between the *HIS4* transcription factors and the enzymes that catalyze meiotic recombination. (*iii*) The binding of transcription factors may create an open chromatin structure that favors both transcription and meiotic recombination.

We prefer the last alternative. Since the oligonucleotide with two RAP1 binding sites and the telomeric oligonucleotide have hotspot activity, any model involving a specific interaction of BAS1, BAS2, and RAP1 with the recombination machinery is unlikely. In addition, by the last model, one might expect a general correlation between sequences that stimulate recombination and those that stimulate transcription; we find such a correlation. If the binding sites were fully occupied, the sequences that had hotspot activity in our study cover about 50–60 bp of DNA. This size may represent a lower limit for creating a region of the chromosome that is accessible to the recombination and/or transcription machinery.

Since all of the hotspots that we investigated are likely to have RAP1 binding sites (see below), it is possible that binding of RAP1 creates a chromatin configuration that is particularly favorable for recombination. The binding of RAP1 to the *HIS4* promoter dramatically increases the *in vitro* nuclease sensitivity of chromatin in the upstream region (8) and might also increase accessibility of the chromatin to the nucleases responsible for initiating recombination. Since RAP1 affects DNA bending, formation of DNA loops, and attachment of DNA to the nuclear matrix (reviewed in ref. 13), alternative models for the effect of RAP1 on recombination also exist.

The telomeric sequences created strong hotspots when inserted 5′ of *HIS4* or *ARG4*. Telomeric sequences at the ends of the yeast chromosomes have high levels of mitotic recombination (19), and interstitial telomeric repeats appear to stimulate meiotic recombination in *Caenorhabditis elegans* (20). The homozygous 51-bp telomeric insertion increased the recombination distance between *LEU2* and *HIS4* by about 30 centimorgans (Table 2). Since, averaged over the entire yeast genome, there are about 3 kb per centimorgan (2), the telomeric sequences stimulate meiotic recombination 1000-fold more effectively than an average yeast sequence.

Although the oligonucleotide containing telomeric DNA has no RAP1 binding sites with a perfect match to the published RAP1 consensus binding site (13, 21), there are three sites with matches at 11 of 13 bases (Fig. 2*c*). Although we cannot be sure whether RAP1 recognizes any of these sites, the lack of a perfect match is not a strong argument against this possibility, since the *HIS4* binding site for RAP1 also does not perfectly match the consensus sequence (8). Thus, the hotspot activity of the telomeric sequences may be a consequence of the effects of RAP1. Alternatively, the stimulation could reflect the binding of other telomere-specific proteins or a mechanistically different effect (for example, extensive nicking of telomeric sequences). The stimulation of *HIS4* gene expression by telomeric DNA is likely to represent an effect of RAP1 binding, since a duplication of the RAP1 binding site has a similar effect. Stimulation of gene expression by telomeric sequences in yeast has been reported previously (21, 22).

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