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Genetics

DNA-binding protein RAP1 stimulates meiotic recombination at the *HIS4* locus in yeast

(*Saccharomyces cerevisiae*/recombination hotspot/gene conversion)

MICHAEL A. WHITE, MONIKA WIERDL, PETER DETLOFF, AND THOMAS D. PETES

Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280

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ABSTRACT **In the yeast *Saccharomyces cerevisiae*, as in other eukaryotes, some regions of the genome have a much higher rate of meiotic recombination than others. We show below that the binding of the RAP1 protein to a site upstream of the *HIS4* gene is necessary for a high rate of meiotic (but not mitotic) recombination at this locus. A mutation in the RAP1 binding site at *HIS4* results in a decrease in recombination; overproduction of RAP1 causes an increase in recombination at *HIS4* above wild-type levels.**

Two types of meiotic recombination events occur in all eukaryotes that have been examined, crossovers (reciprocal recombinations) and gene conversions (nonreciprocal recombinations). In a heterozygous diploid (alleles *A* and *a*) yeast strain, a conversion event is signaled by a tetrad with a 1:3 or 3:1 ratio of *A* to *a* spores instead of the normal Mendelian segregation of 2:2 (1, 2). Although the median frequency of gene conversion in *Saccharomyces cerevisiae* is about 5%, mutations at the 5′ end of *HIS4* have a conversion frequency of about 50% (3, 4). For this gene, mutations near the 3′ end of the gene have a lower conversion frequency than those at the 5′ end. Such polarity gradients have also been seen at the *ARG4* locus in yeast and in other fungi (5), and they are usually interpreted as reflecting an initiation site located near the high-conversion end of the gene, from which conversion events are propagated in a distance-dependent manner (2).

Although one study indicated that deletions that remove the promoter of *ARG4* reduce the gene conversion frequency of *arg4* mutations (6), a more recent analysis (7) indicates that a poly(A) region is the *ARG4* recombination hotspot and that the rate of conversion is unrelated to the level of transcription. At *HIS4*, a deletion of 5′ sequences (−146 to −316) reduced the frequency of gene conversion (P.D., M.A.W., M. Strand, and T.D.P., unpublished data). As shown in Fig. 1, the region upstream of *HIS4* contains binding sites for the proteins GCN4, BAS2, and RAP1 (8–10). RAP1 [which is probably the same protein as TUF and GRF (11–14)] binds in front of many yeast genes, acting as either an activator or a repressor of transcription.

Devlin *et al.* (8) identified the binding site for RAP1 at *HIS4* and constructed mutant derivatives of this site (such as *his4-51*) that had no binding activity (Fig. 1). Strains carrying this mutation have a reduced rate of *HIS4* gene expression. Below, we examine the effect of *his4-51* on recombination of mutant alleles within *HIS4*. We show that this mutation reduces the gene conversion frequency for alleles at the 5′ end of *HIS4* and reduces crossovers between *HIS4* and a linked marker. The same mutation, however, has no significant effect on the frequency of mitotic recombination. In addition, we show that overproduction of the RAP1 protein significantly elevates the frequency of meiotic gene conversion. These results strongly support the hypothesis that the highest level of gene conversion at *HIS4* requires the upstream binding of the RAP1 protein.

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MATERIALS AND METHODS

Plasmids. Many of the plasmids contained various mutant derivatives of *HIS4*: (*i*) pC1G17 (3) {*Bam*HI fragment containing the *his4-Sal* mutation [a 4-base-pair (bp) insertion in a *Sal* I restriction site at +467 in *HIS4*] in a *Bam*HI site in YIp5}; (*ii*) pMW35 {*Sau*3A fragment [derived from plasmid pCB600 (8)] with the *his4-51* mutation in a *Bam*HI site of B142 (YIp5 without the *Pvu* II site)}; (*iii*) pPD8 [identical to pMW35 except that the sequences with the RAP1 binding site (from −219 to −182) are inverted with respect to flanking sequences]; (*iv*) pDN4 [mutant allele *his4-lop* (a palindromic insertion in a *Sal* I site of *HIS4*) in a YIp5 derivative (3)]; (*v*) B138 [provided by B. Ruskin and G. Fink of Massachusetts Institute of Technology; *Bam*HI–*Eco*RI fragment with the *his4-713* mutation (located at position +2270) inserted into YIp5]; (*vi*) pMW2 (*Bgl* II fragment with the 3′ end of *HIS4* in a *Bam*HI site of YIp5); and (*vii*) pMW45 [derivative of pMW2 with the RAP1 binding site (5′-CTAGATTGCTAAAC-CCATGCACAGTGACTCTACGT) in a *Bst*EII site]. The plasmid pMW32 contains a *Bam*HI fragment with the *leu2-Bs* allele (a 4-bp insertion at a *Bst*EII site within the coding sequence) in the *Bam*HI site of YIp5. The plasmid I3H3 (15) contains the RAP1 gene on a high-copy-number plasmid (YEp13), whereas the plasmid S158 (provided by S. Kurtz and D. Shore, Columbia University) contains the RAP1 gene on a different high-copy-number vector (B72). The selectable genes on YEp13 and S158 are *LEU2* and *URA3*, respectively.

Yeast Strains. With the exception of strains MW122 and MW126, all strains were derived from the haploid strains AS4 (*α trp1-1 arg4-17 tyr7-1 ade6 ura3*) and AS13 (**a** *leu2-Bs ade6 ura3*) by transformation. In addition to the auxotrophic markers, these strains contain multiple other heterozygous markers between *LEU2* and *CEN3* (16). For most of the strain constructions, we replaced the wild-type *HIS4* sequences with mutant derivatives by using the two-step transplacement procedure (17). Since strains containing the *his4-51* mutation are histidine independent (His$^+$), we monitored the transplacement by Southern analysis; the mutant allele includes a *Sac* I site that is not present in the wild-type strain. In describing these strains, we show the strain name and (in parentheses) the name of the progenitor strain and the plasmid used in the construction; we signify a transplacement with a slash and an autonomous plasmid with a colon. The haploid strains were MW1 (AS13/pC1G17), MW30 (AS4/pMW35), MW31 (MW1/pMW35), MW55 (AS4/pMW45), PD104 (AS4/pPD8), PD105 (MW1/pPD8), DNY9 (AS13/pDN4), MW33 (DNY9/pMW35), PD23 (AS4/B138), PD24 (AS13/B138), MW54 (PD24/pMW45), MB1 (MW30/B138), and MW37 (MW1:B72). In addition to these strains, two other haploids were used: NKY278-1c (*α ura3 lys2 ho*::*LYS2*; ref. 18) and MW13 (a derivative of AS4 with the *leu2-Bs*

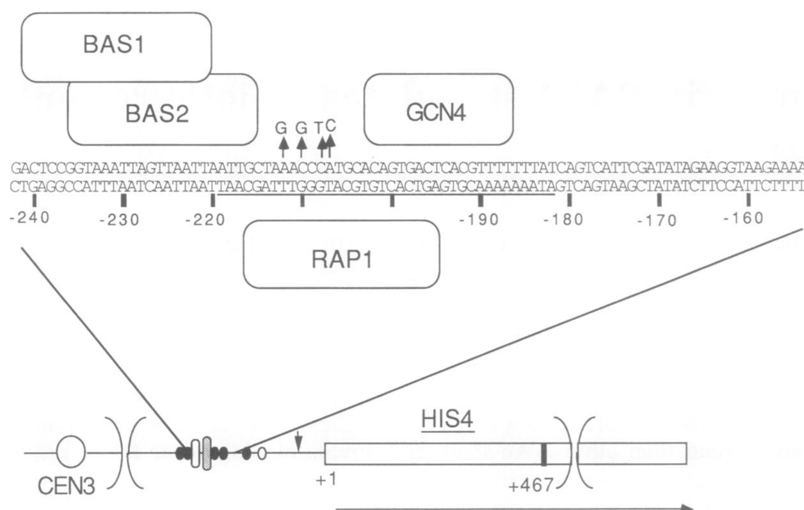

FIG. 1. DNA sequences and protein-binding sites upstream of *HIS4*. The upper part of the figure is based on figure 2 of Devlin *et al.* (8). The BAS1 protein does not bind to DNA directly (9). The base pairs indicated by arrows represent the mutational changes in *his4-51* (8). The sequences underlined represent the inversion in *his4-flp*. On the bottom part of the figure, GCN4 binding sites are indicated by black circles, the TATAA sequence by a white circle, the BAS2 binding site by a white oval, and the RAP1 binding site by a stippled oval. The vertical arrow indicates the mRNA start, the horizontal arrow shows the direction of transcription, and +1 indicates the first base in the ATG codon. The rectangle represents the *HIS4* gene, with the position of the *his4-Sal* mutation shown as a thick vertical line.

mutation and several additional heterozygous restriction sites between *HIS4* and *LEU2*).

The diploid strains used in these studies were derived as follows (× indicating a cross): MW103 (AS4 × MW1), MW114 (MW30 × MW1), MW115 (MW30 × MW31), PD106 (PD104 × PD105), PD92 (AS4 × PD24), DNY11 (AS4 × DNY9), MW118 (MW30 × MW33), MW123 (MW13 × MW1), MW124 (MW123:YEp13), MW119 (MW123:I3H3), MW130 (MW103:S158), MW132 (AS4 × MW37), MW128 (MW115:S158), MS25 (NKY278c × MW1), MW122 (MS25:S158), MW126 (NKY278c × MW37), MB2 (MB1 × MW31), MS1 (PD23 × MW1), and MW141 (MW55 × MW54). The *HIS4* genotypes of these strains are summarized in Table 1.

Methods of Genetic Analysis. Standard procedures for mating and yeast transformation were used (19). Cells were sporulated on plates at 18°C and tetrads were dissected onto plates that were incubated at 32°C; our strains have about two times more meiotic gene conversion at *HIS4* at 18°C than 25°C (3). The resulting colonies were replica-plated to various types of omission media.

Mitotic recombination studies were done with strains MS1 and MB2. These strains were heteroallelic for noncomplementing *his4* mutations and, therefore, were His$^-$. The diploids were streaked to give single colonies on YPD plates (19) and grown for 3 days at 32°C. Individual small colonies were resuspended in water. The appropriate dilution of a fraction of the sample was plated on nonselective (SD-complete) plates (19) to determine the number of cells in the colony, and the remainder of the sample was plated on medium lacking histidine to determine the number of His$^+$ cells. The rate of mitotic recombination was determined from the frequency by the method of Lea and Coulson (20).

Methods of Physical Analysis. We did Southern analysis by standard methods (21), using the plasmid pM45 as a hybridization probe. This plasmid contains a *Sac* I–*Spe* I fragment with the *HIS4* gene inserted into pUC19 (3). RNA was isolated as described by Domdey *et al.* (22) and analyzed by electrophoresis in a formaldehyde/agarose gel. The RNA molecules were transferred to Hybond-N membrane (Amersham) (21) and hybridized to a ^{32}P-labeled 2.8-kilobase (kb) *Bgl* II fragment (derived from plasmid S158) containing the *RAP1* gene. The intensity of the RAP1-specific mRNA was measured by densitometer tracing of autoradiographs and normalized to the A_{260} of the RNA preparation.

Statistical Methods. In comparisons in which all classes of events had more than five representatives, we did a contingency χ^2 analysis. For comparisons in which one or more of the classes had fewer than five events, we determined *P* values with the Fisher exact test, using the STATXACT program from Cytel Software Corp. Any value of *P* less than 0.05 was considered statistically significant.

RESULTS

We observed previously that mutant alleles located near the 5′ end of the *HIS4* gene have a very high frequency of meiotic gene conversion (3, 4), about 50% of unselected tetrads. Alleles at the 3′ end convert at a frequency of about 20% (ref. 4 and unpublished observations). One interpretation of this result is that conversion events initiate near the 5′ end of the gene. As mentioned in the introduction, we previously observed that a deletion of the sequences from −146 to −316 (Fig. 1) reduced the level of meiotic gene conversion of alleles near the 5′ end of *HIS4*. Since the strongest "footprint" in this region is the result of binding of the RAP1 protein (8), we examined the effects of this interaction on the frequency of meiotic gene conversion. All strains used in this study (with the exception of MW122 and MW126) are isogenic.

A Mutation of the RAP1 Binding Site Reduces the Frequency of Meiotic Gene Conversion. Devlin *et al.* (8) constructed a mutation of the *HIS4* RAP1 binding site (*his4-51*; Fig. 1) that eliminates the binding of the protein *in vivo* and *in vitro*. Strains containing this mutation are His$^+$. The frequency of meiotic gene conversion of *his4-Sal* (a 4-bp insertion located at position +467 relative to the initiating codon) is 31% in a strain with the wild-type RAP1 binding site (strain MW103; Table 1). In a diploid strain (MW115) homozygous for *his4-51* and heterozygous for *his4-Sal*, the conversion frequency of *his4-Sal* is reduced to 12% (Table 1). The difference in conversion frequencies is highly significant ($\chi^2 = 25$; $P < 0.001$). In a strain heterozygous for the binding site (MW114), the conversion frequency is also significantly reduced, specifically in the $1^+:3^-$ class of tetrads ($\chi^2 = 24$; $P < 0.001$). One interpretation of these data is that the chromosome with the wild-type RAP1 binding site is an effective recipient of information during a meiotic gene conversion event; other

Table 1. Number and frequency of aberrant segregation events at the *HIS4* locus

Strain	Relevant genotype	Plasmid (*RAP1*?)*	Conversion events				PMS events†	Total tetrads	Aberrant segregation frequency,‡ %
			$3^+:1^-$	$1^+:3^-$	$4^+:0^-$	$0^+:4^-$			
MW103§	*his4-Sal* / *HIS4*		31	50	2	6		291	31
MW114	*his4-Sal* / *his4-51 HIS4*		30	12	2	0		249	18
MW115	*his4-51 his4-Sal* / *his4-51 HIS4*		11	19	0	0		246	12
PD106	*his4-flp his4-Sal* / *his4-flp HIS4*		37	50	3	3		301	31
DNY11§	*his4-lop* / *HIS4*		23	6	1	0	79	312	35
MW118	*his4-51 his4-lop* / *his4-51 HIS4*		7	2	0	1	49	325	18
PD92§	*his4-713* / *HIS4*		23	37	2	1		293	22
MW141	3′ site *his4-713* / 3′ site *HIS4*		25	28	0	0	4	264	22
MW119	*his4-Sal* / *HIS4*	I3H3 (yes)	57	67	5	6		354	40
MW124	*his4-Sal* / *HIS4*	YEp13 (no)	42	55	1	0		353	29
MW130	*his4-Sal* / *HIS4*	S158 (yes)	57	59	4	2		352	35
MW132	*his4-Sal* / *HIS4*	B72 (no)	40	55	0	3		371	26
MW128	*his4-51 his4-Sal* / *his4-51 HIS4*	S158 (yes)	7	16	0	0		284	8
MW122¶	*his4-Sal* / *HIS4*	S158 (yes)	12	25	2	4		305	14
MW126¶	*his4-Sal* / *HIS4*	B72 (no)	3	3	0	0		186	3

*In those strains containing a plasmid, the name of the plasmid is indicated; in parentheses, "yes" indicates that the plasmid has the *RAP1* gene, and "no" indicates a plasmid without this gene.

†PMS (post-meiotic segregation) events represent tetrads in which one or more spore colonies show sectored growth on plates lacking histidine.

‡This number represents the sum of the conversion and post-meiotic segregation tetrads divided by the total.

§These data have been reported previously (3, 4).

¶These strains have a different genetic background (NKY278-1c × AS13) than any of the other strains used in our study.

putative recombination initiation sites in yeast have similar effects (2, 6).

The *his4-51* mutation also significantly reduces the frequency of meiotic gene conversion of *his4-lop*, a palindromic insertion of 26 bp at +467 that has high post-meiotic segregation (2). The frequency of aberrant segregation is reduced from 35% in DNY11 (wild-type binding site) to 18% in MW118 (mutant binding site). The *his4-51* mutation significantly (χ^2 values of 10 or more; $P < 0.01$) reduces both gene conversion events and post-meiotic segregation events (Table 1). Since post-meiotic segregation events reflect heteroduplex formation between mutant and wild-type genes (1, 2), this result suggests that sequences in the RAP1 binding site are necessary for the highest levels of heteroduplex formation in *HIS4*.

The binding site of RAP1 is not symmetric (13, 14). To determine whether the orientation of this site affects the frequency of meiotic recombination, we constructed a strain (PD106) in which the binding site was inverted relative to the 5′ end of the gene (*his4-flp*/*his4-flp*). The frequency of gene conversion of *his4-Sal* is identical to that in the strain with the wild-type orientation of the binding site.

A Mutation of the RAP1 Binding Site Reduces the Frequency of Meiotic Crossing-over. We also examined the effect of the *his4-51* mutation on the frequency of crossing-over between *LEU2* (a heterozygous marker located about 25 kb centromere-proximal to *HIS4*) and *HIS4*. Crossovers in strains containing two heterozygous linked markers are signaled by tetratype and nonparental ditype asci. Strains homozygous for the *his4-51* mutation had significantly reduced ($P = 0.002$ or less, Fisher exact test) levels of crossing-over between *HIS4* and *LEU2* (Table 2). The orientation of the RAP1 binding site had no effect on the frequency of crossovers (comparison of PD106 and MW103, $P = 0.5232$).

Overproduction of the RAP1 Protein Elevates the Frequency of Gene Conversion at *HIS4*. Extra copies of the *RAP1* gene were introduced into yeast by using the multicopy plasmid I3H3 (YEp13 vector; ref. 15) or S158 (B72 vector, provided by S. Kurtz and D. Shore). Northern analysis showed that the level of *RAP1* message was increased approximately 3-fold in strains with I3H3 or S158 compared to strains with the vectors without the insert. The frequency of gene conversion of the *his4-Sal* mutation was significantly increased in the strains carrying the *RAP1*-multicopy plasmids as compared with the controls (Table 1); by contingency χ^2 analysis, the smallest χ^2 value for these pairwise comparisons was 5 ($P < 0.05$). Although the conversion frequencies were increased by only one-third in strains with high *HIS4* conversion, in a genetic background with low *HIS4* conversion, the frequency of conversion was elevated 4-fold (MW122 compared to MW126). Conversion rates of other heterozygous markers (*LEU2*, *TRP1*, *TYR7*, *ARG4*, and *MAT*) were not affected by

Table 2. Number of tetrads with parental ditype, nonparental ditype, or tetratype segregation patterns for the heterozygous *HIS4* and *LEU2* markers

Strain	Relevant genotype	Segregation pattern of *LEU2–HIS4** PD	NPD	T	Map distance, cM†
MW103	*his4-Sal* / *HIS4*	101	1	90	25
MW115	*his4-51 his4-Sal* / *his4-51 HIS4*	137	4	64	21
DNY11	*his4-lop* / *HIS4*	132	10	119	34
MW118	*his4-51 his4-lop* / *his4-51 HIS4*	188	1	112	19
PD106	*his4-flp his4-Sal* / *his4-flp HIS4*	103	4	92	29
MW130	*his4-Sal* + S158 / *HIS4*	119	4	96	27
MW132	*his4-Sal* + B72 / *HIS4*	144	4	114	26

*Only those tetrads in which both the *HIS4* and the *LEU2* markers segregated 2:2 are shown. For linked markers, parental ditype tetrads (PD) usually indicate noncrossover (or two-strand double-crossover) tetrads, nonparental ditype tetrads (NPD) represent four-strand double-crossover tetrads, and tetratype tetrads (T) signify single- (or three-strand double-) crossover tetrads (2).
†Calculated by the equation of Perkins (23); cM, centimorgans.

overproduction of RAP1 (data not shown). The frequencies of crossovers between *HIS4* and *LEU2* were not significantly affected by overproduction of the RAP1 protein (Table 2).

The RAP1 Binding Site Does Not Induce High Levels of Meiotic Recombination in All Contexts. We next examined whether insertion of the RAP1 binding site downstream of *HIS4* would increase the gene conversion frequency of a mutant allele located at the 3′ end of the gene. The *his4-713* allele is a 1-bp insertion located at position +2270 (24). The frequency of gene conversion of this allele in a strain (PD92) with the wild-type arrangement of the RAP1 binding site is 22% (Table 1). We constructed a second strain (MW141) that was heterozygous for *his4-713* and, in addition, homozygous for an insertion of the RAP1 binding site (sequences −219 to −194) into a *Bst*EII site located at +2649. This binding site is located outside the *HIS4* coding region and about 400 bp from the heterozygous mutant allele. Although the RAP1 binding site stimulates gene conversion over a distance of over 600 bp at the 5′ end, no stimulation of gene conversion was observed for *his4-713* in the presence of the 3′ RAP1 site. We conclude that the RAP1-mediated stimulation of meiotic recombination is context dependent.

A Mutation of the RAP1 Binding Site Does Not Affect the Frequency of Mitotic Gene Conversion. To study the effect of the mutation in the RAP1 binding site on mitotic recombination, we examined the diploid strains MS1 and MB2. The strain MS1 has the wild-type RAP1 binding site, and the strain MB2 is homozygous for the *his4-51* mutation. Each of these strains contains one copy of *HIS4* with the *his4-Sal* mutation and the other copy with the *his4-713* mutation (24). Since these mutations do not complement, the diploid is phenotypically His$^-$. In *S. cerevisiae*, prototrophic derivatives that arise during mitotic growth of a heteroallelic diploid reflect mitotic gene conversion events (25). Since the rates of conversion were similar in the two strains (Table 3), we conclude that the DNA sequences in the wild-type RAP1 site do not influence mitotic recombination.

DISCUSSION

The simplest interpretation of the observation that the *his4-51* mutation reduces gene conversion and that overproduction of the RAP1 protein stimulates conversion is that the binding of the RAP1 protein to the RAP1 site is required for the highest level of meiotic recombination at the *HIS4* locus. The stimulation of recombination observed when RAP1 is overproduced is not likely to be an indirect effect of this RAP1 protein influencing the level of recombination enzymes, since no stimulation is observed at *HIS4* when the RAP1 site is mutated (strain MW128, Table 1). The observed stimulation was more dramatic in strains with a low level of conversion at *HIS4* than in strains with a high level (Table 1). This result suggests that in some strains the level of RAP1 may limit the amount of gene conversion, whereas in other strains a different gene product may be rate limiting.

Table 3. Rates of mitotic recombination (conversion events per cell division) at the *HIS4* locus in strains with and without the RAP1 binding site

Strain MS1 (wild-type RAP1 binding site) No. of independent cultures	Recombination rate × 10^4	Strain MB2 (mutant RAP1 binding site) No. of independent cultures	Recombination rate × 10^4
25	0.9	15	1.1
24	1.9	18	0.8
24	1.4	20	1.2
20	1.4	19	1.1
24	1.3	18	1.2
Average for MS1	1.4	Average for MB2	1.1

The rates were estimated by determining the frequency of His$^+$ cells in a number of independent cultures and using the methods of Lea and Coulson (20).

Most meiotic gene conversion events in yeast appear to be the result of asymmetric heteroduplex formation followed by repair of the resulting mismatch (1, 2); mismatch repair that uses the invading strand as a template (conversion-type repair) results in a conversion event, whereas repair that uses the invaded strand as a template (restoration-type repair) results in 2^+:2^- segregation (2). Thus, the reduction of gene conversion observed in strains with the *his4-51* mutation could reflect either a reduction in the frequency of the initiation of heteroduplex formation or an increase in the amount of restoration repair, or both factors. The observation that the frequency of recombination for the high-post-meiotic-segregation *his4-lop* allele is significantly reduced by *his4-51* suggests that at least part of the effect of the mutation is on the initiation of heteroduplex formation.

The mechanism by which the RAP1 binding affects the initiation and/or resolution of meiotic recombination event at *HIS4* is unknown. The binding of RAP1 has been shown to affect DNA bending (26), DNA loop formation (27), association of DNA with the nuclear matrix (27), and nucleosome positioning (8). Any of these factors could affect the accessibility of the *HIS4* region to the enzymes that catalyze meiotic recombination. Alternatively, it is possible that RAP1 may directly interact with recombination proteins.

The frequency of certain mitotic recombination events in yeast is stimulated by transcription (28, 29). It is unlikely that the effect of the RAP1 protein on meiotic recombination is related to its effects on *HIS4* transcription. Although strains with the *his4-51* mutation have reduced transcription under certain growth conditions (8), the rate of transcription is reduced less severely than in strains with a promoter deletion that has no effect on the rate of meiotic recombination (M.A.W., P.D., M. Strand, and T.D.P., unpublished data). The *his4-51* mutation had no significant effect on mitotic recombination at the *HIS4* locus. This result indicates that small changes in the level of transcription do not necessarily affect the rate of mitotic recombination.

When we moved the RAP1 binding site downstream of *HIS4*, no stimulation of gene conversion was observed for alleles located at the 3′ end of the gene. This result indicates that this site is not sufficient to stimulate recombination in all contexts, although it should be pointed out that we do not know whether the RAP1 protein binds to the binding site in its new context. The simplest interpretation of the data is that the RAP1 protein interacts with a second protein that is bound to a different nearby site to initiate recombination. Despite the lack of effect of the binding site at the 3′ end of *HIS4*, there is some evidence that RAP1 binding may be able to affect recombination rates at other loci. A sequence required for high rates of recombination between *LEU2* and the centromere (30) contains two reasonably good (11 of 13 bp) matches to the RAP1 binding site consensus sequence (13, 14). An insertion of a fragment of pBR322 that stimulates meiotic recombination (16) has a similar match to the RAP1 consensus sequence.

Prior to this study, only large (greater than 50-bp) deletions were observed to affect the frequency of meiotic recombination in *S. cerevisiae*. In *Schizosaccharomyces pombe*, however, a 1-bp change in the *ade6* gene (the M26 mutation) results in strong stimulation of both gene conversion and crossing-over (31–34). As with the *HIS4* and *ARG4* (6) hotspots, if the M26 mutation is heterozygous, the chromosome with the hotspot behaves as a recipient of genetic information. This result is consistent with the hotspot's acting as a site for a double-strand break or as a site for generating a single-stranded gap. There are two other similarities between the M26 hotspot and the RAP1 binding site at *HIS4*: the M26 sequence does not function as a hotspot in other chromosomal contexts (A. Ponticelli and G. R. Smith, personal communication), and a protein binds to the M26 sequence but not the wild-type ADE6 sequence (W. P. Wahls and G. R. Smith, personal communication). Thus, in two different fungi, meiotic recombination is stimulated by binding of a specific protein at a specific site.

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