Analysis of a Recombination Hotspot for Gene Conversion Occurring at the *HIS2* Gene of *Saccharomyces cerevisiae*

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ABSTRACT

The properties of gene conversion as measured in fungi that generate asci containing all the products of meiosis imply that meiotic recombination initiates at specific sites. The *HIS2* gene of *Saccharomyces cerevisiae* displays a high frequency of gene conversion, indicating that it is a recombination hotspot. The *HIS2* gene was cloned and sequenced, and the cloned DNA was used to make several different types of alterations in the yeast chromosome by transformation; these alterations were used to determine the location of the sequences necessary for the high levels of meiotic conversion observed at *HIS2*. Previous work indicated that the gene conversion polarity gradient is high at the 3′ end of the gene, and that the promoter of the gene is not necessary for the high frequency of conversion observed. Data presented here suggest that at least some of the sequences necessary for high levels of conversion at *HIS2* are located over 700 bp downstream of the end of the coding region, extend over (at least) several hundred base pairs, and may be quite complex, perhaps involving chromatin structure. Additional data indicate that multiple single base heterologies within a 1-kb interval contribute little to the frequency of gene conversion. This contrasts with other reports about the role of heterologies at the *MAT* locus.

GENETIC recombination occurs at high frequency in meiotic cells, and in most organisms is essential for proper chromosome segregation during the reductional division in meiosis I. The problem of how homologous chromosomes find each other and begin recombination is one which still remains largely unanswered. Over the last few years, a large number of genes have been found in the yeast *Saccharomyces cerevisiae* that appear to be required for the initiation of recombination and pairing in meiosis (*e.g.*, MALONE 1983; KLAPHOLZ *et al.* 1985; ROCKMILL and ROEDER 1988; MENEES and ROEDER 1989; HOLLINGSWORTH and BYERS 1989; MALONE *et al.* 1991). One would imagine that at least some of these genes code for functions that are involved in bringing chromosomes together, acting on DNA, and beginning exchange. Likewise, two loci have been examined in *S. cerevisiae* to determine whether (and if so, how) specific *cis*-acting sequences might also be involved in recombination (NICOLAS *et al.* 1989; WHITE *et al.* 1991).

Recombination has two different genetic manifestations: crossing over generates reciprocal recombinants in equal amounts, while gene conversion leads to nonreciprocal products. Because of the need to observe all chromatids involved in the exchange event to detect conversion, most of the data relating to gene conversion have been obtained from fungi whose haploid meiotic products are contained in an ascal sack. Genetic analysis of the spores in the asci by microdissection allows an unbiased determination of the amount and type of conversion that occurs.

Several properties of gene conversion suggest that it does not occur randomly along chromosomes. First, different loci display very different frequencies of conversion (or more completely, aberrant segregation); in the yeast *S. cerevisiae* typical variations range from 0.6% to 18% (FOGEL *et al.* 1978). Second, a monotonic gradient of conversion frequencies usually exists across a gene; more rarely a conversion gradient may have a more complex pattern (WHITEHOUSE 1982). Usually, however, markers at one end of the gene display higher frequencies of aberrant segregation than markers at the other end of the gene. In the yeast *S. cerevisiae* (with rare exceptions), the frequencies seem to depend upon the position of the allele within the gene, not on the specific allele itself (FOGEL *et al.* 1978). These observations are easily understandable if there are specific initiation sites where recombination (*i.e.*, gene conversion) can begin, and if the events which occur at these sites extend for varying distances with the probability of an event reaching a specific allele being inversely related to the distance between the allele and the initiation site. Studies on the coconversion of several alleles in the same gene provide further support both for the concept of specific initiation sites and for the distance dependence of gene conversion (FOGEL *et al.* 1978; SCHULTES and SZOSTAK 1991).

Recently, two loci in *S. cerevisiae* (*ARG4* and *HIS4*) have been examined using both classical and molecular genetics to determine whether meiotic recombination initiation sites really do exist. SZOSTAK and colleagues

investigated gene conversion at the *ARG4* locus of yeast (NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990). They found that a region at the 5′ end of the gene is necessary for the normal conversion frequency observed at this gene; this is consistent with the direction of the polarity gradient. A marker which normally converted at a frequency of 7.4% displayed a conversion frequency of 2% when an *Nsp*HI fragment of 142 bp containing part of the promoter region was deleted. Deleting another 573 bp upstream of the *Nsp*HI fragment (for a total deletion of 815 bp) reduced the frequency to 0.8%. Taken together, the analysis of the deletions supported the hypothesis that a region of several hundred bp located upstream of the coding sequence in the promoter region was required for the normal level of gene conversion at *ARG4*. More recently, these investigators have found that deletion of a poly(A) sequence in the promoter region reduces conversion from 7.4 to 3.3% (SCHULTES and SZOSTAK 1991). PETES and colleagues have also found that sequences in the promoter region of the *HIS4* gene are important for high levels of conversion (WHITE *et al.* 1991); alteration of a Rap1 protein binding site located in the *HIS4* promoter region lowers conversion substantially (from 31 to 12%). Interestingly, the situation may be different in the yeast *Schizosaccharomyces pombe*, since the M26 hotspot for meiotic recombination is located within the coding sequence of the gene (PONTICELLI *et al.* 1988; PONTICELLI and SMITH 1992).

To help determine what, if any, *cis*-acting DNA sequences were generally responsible for gene conversion in yeast, we examined the *HIS2* locus. We chose the *HIS2* gene on chromosome *VI* because, at the time we started the work, it was reported to have the highest frequency of conversion of any gene that had been examined in yeast (FOGEL *et al.* 1978); for this reason we refer to it as a recombination hotspot. Many properties of gene conversion have been explained in molecular terms by popular models for genetic recombination, such as the single strand invasion model of MESELSON and RADDING (1975), or the double strand break model of SZOSTAK *et al.* (1983). However, one property of gene conversion which seems to be model independent is its dependence upon distance. Thus, a locus exhibiting a high frequency of conversion would be closer to an initiation site than a gene with a lower frequency, if, as it appears in *S. cerevisiae*, conversion does not occur randomly along the chromosome. To determine what was responsible for the high frequency of gene conversion at *HIS2*, we cloned the gene and several kilobases (kb) of surrounding DNA. We found that the polarity gradient is higher at the 3′ end of the gene and lower at the 5′ end; furthermore, deletion of the promoter region does not reduce gene conversion (MALONE *et al.* 1992). We have made alterations in the cloned DNA, reintroduced them into yeast by transformation, and analyzed the effect of each alteration on recombination. Our data suggest that the hotspot for conversion at the *HIS2* locus may be somewhat different than hotspots at *ARG4* and *HIS4*; at least some of the sequences required for high levels of conversion at *HIS2* appear to be located more than 700 bp downstream of the gene, and may be scattered over several hundred to several thousand bases. The latter observation suggests that higher level chromosome structure may play a role. A similar conclusion, that chromosome context plays a role in recombination hotspots, has recently been made for the M26 hotspot in *S. pombe* (PONTICELLI and SMITH 1992).

MATERIALS AND METHODS

Yeast and bacterial strains: All yeast strains used in the experiments described in this paper are shown in Table 1. The parents for all diploids used for measuring gene conversion are defined in the legend to Table 1. Note that strain RM182-55C is the product of three backcrosses of *cdc14 cly3* segregants with RM113-4B to acquire the outside markers *cdc14* and *cly3*. (The sequence of the *HIS2* allele in RM182-55C is identical to the *HIS2* allele in RM96-15A.) The bacterial strain RK1448 (COOL and MALONE 1992), a *recA*$^-$ strain with good transformation efficiency, was used for DNA manipulations. All plasmids used are described in Table 1. The genotypes for all diploids can be derived from the genotypes of the haploid parents. At least 4, and usually 5, other loci were checked for segregation in each cross done to measure gene conversion at the *HIS2* locus; this was done in order to eliminate false tetrads.

Cloning the *HIS2* gene and sequencing the 2.9-kb *Eco*RI fragment: A YEp24 library of *S. cerevisiae* [generously provided by C. Falco (DuPont)] was used to transform RM113-4B, and His$^+$Ura$^+$ transformants were selected. Two overlapping clones were isolated: pH21 (a restriction map is shown in Figure 1) and pH22; they share a common 2.9-kb *Eco*RI fragment which complemented *his2-1*, integrated at the chromosomal *HIS2* locus (see Results), and proved to contain the *HIS2* gene. The sequence (see Figure 3) of the *HIS2* allele in RM96-15A was determined (after gap rescue) by dideoxy sequencing as previously described (COOL and MALONE 1992) using the Sequenase kit (U.S. Biochemical Corp.). The sequence of the *HIS2* allele found in pH21 was identical.

Mutations in the *HIS2* gene: All mutations (except the *his2-1* allele, see below) used to analyze recombination were created *in vitro* in the *HIS2* background and then used to replace the wild-type gene by one- or two-step gene replacement (ROTHSTEIN 1991). The mutations' locations are shown in Figure 2. Lower case restriction site names indicate that a site was eliminated; upper case names indicate that a restriction site was created. The mutations (locations in parentheses) were constructed as follows: *his2-NSI* (bp +6), a single base insertion of T created by oligonucleotide-directed *in vitro* mutagenesis that creates an *Nsi*I site; *his2-2* (bp +290), a single base substitution (G → A) created by UV mutagenesis; *his2-BGL* (bp +301), a single base substitution (G → T) created by oligonucleotide-directed mutagenesis that creates a *Bgl*II site; *his2-bam* (bp +402), a 4-base insertion created by filling in a *Bam*HI site; *his2-bcl* (bp +509), a 4-base insertion created by filling in a *Bcl*I site; *his2-xho* (bp +749), a 4-base insertion created by filling in a *Xho*I site; *his2-acc* (bp +885), a 2-base insertion created by filling in an *Acc*I site; and *his2-hpa* (bp +947) created by inserting an 8-bp *Cla*I linker in a *Hpa*I site.

The *his2-1* allele was recovered from strain RM113-4B by gap rescue (ROTHSTEIN 1991); this is the only allele we found in extant *his2* yeast strains. Allelism tests with over a dozen *his2*

TABLE 1
Yeast Strains and plasmids

A. Yeast strains

Diploid	Relevant Genotype
RM96-15A	**a** *HIS2 ura3-13 met2-1 trp1-1*
RM113-4B	α *his2-1 lys2-1 leu2-3,112 ura3-1 leu1-c lys5-c met2-1 met4 aro2*
RM182-55c	α *HIS2 cly3 cdc14-1 lys2-1 ade5 ade2-1 can1^r ura3-1*
JL4-7	α/**a** *his2-1-D99 / HIS2-D99*
JL4-9	α/**a** *his2-1-D82 / HIS2-D82*
JL4-15	α/**a** *his2-1-D76 / HIS2-D76*
LL2-34	**a**/α *his2-xho-ΔAha / HIS2-ΔAha*
RM101	**a**/α *CDC his2-1 CLY3 / cdc14-1 HIS2 cly3*
RM102	**a**/α *his2-hpa-ΔHX / HIS2-ΔHX*
RM167	**a**/α *CDC14 his2-2 CLY3 / cdc14-1 HIS2 cly3*
RM173	α/**a** *his2-1::URA3* (insertion at *Hin*dIII site) / *HIS2::URA3* (insertion at *Hin*dIII site)
RM174	α/**a** *his2-1::Tn9#16 / HIS2::Tn9#16*
RM175	α/**a** *his2-1 / HIS2::URA3*
RM183	α/**a** his2-1 / *HIS2*
RM187	α/**a** *his2-xho::TUBA66 / HIS2::TUBA66*
RM188	α/**a** *his2-xho::TUBA98 / HIS2::TUBA98*
RM193	α/**a** *CDC14 his2-390 CLY3 / cdc14-1 HIS2 cly3*
RM196	**a**/α *CDC14 his2-xho CLY3 / cdc14-1 HIS2 cly3*
SK2-11	α/**a** *his2-1::TUBA110 / HIS2::TUBA110*
SK2-42	α/**a** *his2-1::TUBA117 / HIS2::TUBA117*
SK2-61	α/**a** *his2-1::TUBA66 / HIS2::TUBA66*
SK2-91	α/**a** *his2-1::TUBA98 / HIS2::TUBA98*
SK3-151	**a** Δ*his2* *lys2-1::HIS2* (2.9-kb *Eco*RI fragment) / α Δ*his2* *LYS2::his2-xho* (2.9-kb *Eco*RI fragment)
SK3-231	**a** Δ*his2* *lys2-1::HIS2* (5.2-kb *Bgl*II fragment orientation 1) / α Δ*his2* *LYS2::his2-xho* (5.2-kb *Bgl*II fragment orientation 1)
SK3-232	**a** Δ*his2* *lys2-1::HIS2* (5.2-kb *Bgl*II fragment orientation 2) / α Δ*his2* *LYS2::his2-xho* (5.2-kb *Bgl*II fragment orientation 2)

B. Plasmids

Name	Description
p3-L6	YEp24 + 14-kb *Sau*3A partial fragment containing *LYS2*
pH21	YEp24 + 10.2-kb *Sau*3A partial fragment containing *HIS2*
pHSS6	Cloning vector for shuttle mutagenesis
pUC18P°	pUC18 with the *Pst*I site in polylinker removed
pJL5	pUC18P° containing 1.9-kb *Eco*RI fragment upstream of *LYS2*
pJL7	pJL5 with 2.9-kb *Eco*RI fragment inserted into *Pst*I site
pRM66	pHSS6 + 5.2-kb *Bgl*II fragment containing *HIS2* inserted at *Bam*HI site
pRM94	pBR322 containing 2.7 *Hin*dIII fragment downstream from *LYS2*
pRM97	pRM94 with 5.2-kb *Bgl*II fragment inserted into *Bgl*II site
pRM98	Identical to pRM97 except opposite orientation of *HIS2* fragment
pSK3	pUC18 + 1.1-kb *Hin*dIII fragment containing *URA3*
pSK5	pSK3 + 5.5-kb *Bgl*II fragment containing *HIS2* from pRM66

The diploids RM101, RM167, and RM196 are all derived from the haploid parents RM96-15A and RM182-55C after appropriate transformations. The diploids RM102, RM173, RM174, RM175, and RM183, JLA4-7, JL4-9, JL4-15, LL2-34, SK2-11, SK2-42, SK2-61, SK2-91, SK2-256, SK3-151, SK231, and SK232 are all derivatives of the haploid parents RM96-15A and RM113-4B after appropriate transformations. Plasmid constructions are described in MATERIALS AND METHODS. Plasmids pRM6, pRM7, pRM9, pRM13, pRM14, pRM22, pRM23, pRM24, pRM26 are subclones (derived from pH21) of the original cloned 10.2-kb yeast fragment and are described in Figure 1.

strains from other laboratories indicated that all of them contain the *his2-1* allele, or at least an allele which does not recombine with it (data not shown). (Generally these strains were only denoted as *his2* with no allele described.) The *his2-1* allele was used for the analysis of insertions (which was done prior to the sequencing of the gene), and for analysis of several of the deletions (see below). After the prototrophic *HIS2* gene was sequenced, we sequenced the *his2-1* allele and found it to contain eight base changes. These are: +191, C → T, Thr to Ser; +252, T → C, Tyr to Tyr; +362, T → C, Ile to Thr; +390, T → A, His to Gln; +469, C → G, His to Asp; +745, C → G, Arg to Gly; +906, T → C, Tyr to Tyr; and +943, C → G, Leu to Val. The only mutation which conferred a His$^-$ phenotype is the change at +390; none of the others (singly, or in several combinations) conferred a His$^-$ phenotype (data not shown). This observation is consistent with the genetic data on conversion (see RESULTS) which indicate that the *his2-1* allele behaves like a single mutation. We confirmed the His$^-$ phenotype

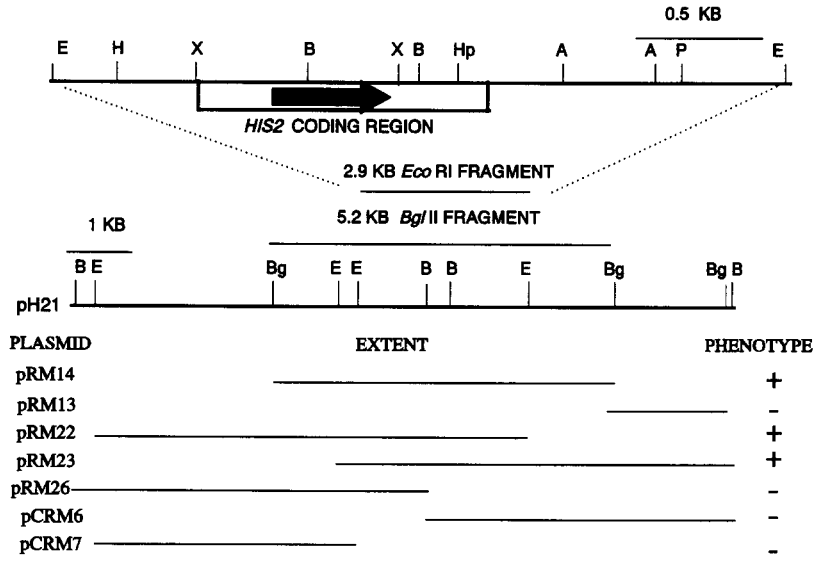

FIGURE 1.—The restriction map of the clone containing the *HIS2* gene isolated in pH21 is shown in the middle of the figure. Above the map of the original clone is an expanded view of the 2.9-kb *Eco*RI fragment containing the *HIS2* gene. (For evidence to support the location of the *HIS2* gene, see text). A, *Aha*III; B, *Bam*HI; E, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII; HP, *Hpa*I; P, *Pst*I; X, *Xho*I. Restriction sites are shown to scale. Below the restriction map is the complementation of the *his2-1* mutation with various subclones of pH21. The data indicate that *HIS2* must be located in the 2.9-kb *Eco*RI fragment.

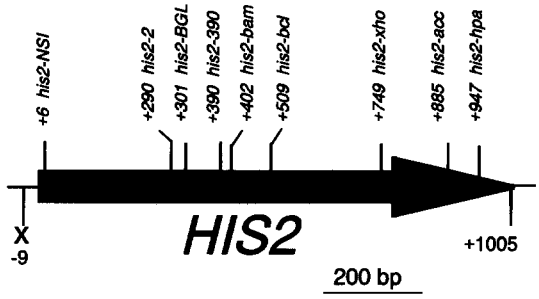

FIGURE 2.—Summary of the locations of the *his2* mutations used to monitor recombination in this study. The *HIS2* coding region extends from base +1 to base +1005 (see Figures 1 and 3). Letters refer to restriction sites as in Figure 1.

conferred by the +390 change by recreating the +390 mutation by oligonucleotide-directed mutagenesis in the *HIS2* DNA and placing the mutation in the chromosome of a *HIS2* strain; the strain became His$^-$. We refer to this allele as *his2-390*. We confirmed that the +390 mutation was the only change from the wild type sequence by sequencing from bp +1 to bp +1100.

Gene conversion measured in the diploid strain containing *his2-390* allele indicated that the presence or absence of the seven silent mutations found in the *his2-1* allele has no significant effect on gene conversion (see RESULTS).

Insertions in the HIS2 region: Three types of insertions were constructed in the *HIS2* region and transplaced back into the yeast chromosome for analysis of effects on gene conversion. All insertions initially were made in clones containing the *HIS2* gene. The 1.16-kb *Hin*dIII fragment containing *URA3* was inserted into the *Hin*dIII site at position −359. The resulting 1.7-kb *Eco*RI-*Xho*I fragment was moved into yeast by one-step gene replacement (ROTHSTEIN 1991). A number of insertions of a 5.5-kb miniTn3 transposon containing *URA3*, *amp*^{r}, and *lacZ* were isolated in the 2.9-kb *Eco*RI fragment containing *HIS2* (Figure 1), and transformed into yeast, according to the protocol of SEIFERT *et al.* (1986), who constructed this "shuttle mutagenesis" system. Briefly, the 2.9-kb *Eco*RI fragment containing the insertion was excised by *Not*I and the isolated linear DNA transformed into yeast spheroplasts selecting for Ura$^+$ cells. We refer to the 5.5-kb insertions as "TUBA" insertions (for miniTn*3*, *URA3*, **B**-galactosidase, **A**mpicillin resistant). A Tn*9* insertion (2.6 kb) was constructed by infecting RK1448 containing pH21 with a defective lambda phage containing Tn*9* (provided by PHIL MATSUMURA, University of Illinois, Chicago). After 60 min of growth, plasmid DNA was isolated by CsCl centrifugation and used to transform RK1448, selecting for Amp^r and Cam^r. All such transformants contained pH21 with Tn*9* inserted. We focused on the analysis of an insert (#16) located in the interesting region between the *Pst*I site (at +1834) and the right *Eco*RI site (+2284). The 2.9-kb *Eco*RI fragment containing Tn*9*#16 was subcloned into a *URA3* integrating vector and placed into the yeast chromosome by two step gene replacement (ROTHSTEIN 1991). The locations of all insertions were initially determined by restriction mapping and subsequently by DNA sequencing. All insertions placed into yeast were confirmed by Southern analysis.

Deletions in the *HIS2* region: All deletions initially were made in clones containing the *HIS2* gene. Three types of deletions were examined. First, the *Aha*III deletion removed the 382-bp *Aha*III fragment located between bp +1349 and +1731 in the *Hin*dIII-*Pst*I fragment containing the *HIS2* gene (Figure 1). The deleted *Hin*dIII-*Pst*I fragment was then subcloned into an *URA3* integrating vector. This plasmid was transformed into yeast and the deletion placed into the chromosome by two-step gene replacement. Second, a series of deletions averaging 600 bp around the *Pst*I site (+1834) was constructed by exonuclease III (ExoIII)/S1 treatment (GUO and WU 1983) after *Pst*I digestion of the cloned 2.9-kb *Eco*RI fragment in pUC18P^o. The 1.16-kb *URA3* *Hin*dIII fragment was inserted into each plasmid containing a deletion, and these were used as an integrating vectors. Three of these deletions were placed in the yeast chromosome by two-step gene replacement. The end points of these deletions were determined by sequencing. Third, a deletion of the *Hin*dIII-*Xho*I fragment located between −359 and −9, which deletes the promoter region of the *HIS2* gene, was constructed (MALONE *et al.* 1992). [The *Hin*dIII-*Xho*I fragment must contain the promoter because a 2.2-kb *Hin*dIII-*Pst*I (Figure 1) subclone is sufficient to confer a His$^+$ phenotype on a cell deleted for the entire 2.9-kb *Eco*RI fragment. Thus, the sequences necessary for transcription of the *HIS2* gene must be located to right of the *Hin*dIII site shown in Figure 1.] Finally, the *HIS2* sequences were deleted from chromosome *VI* for the experiments where *HIS2* was moved to chromosome *II*. This was done by removing both the 0.4- and 2.9-kb *Eco*RI fragments from the 5.2-kb *Bgl*II fragment (Figure 1) to form plasmid pSK5-1. The resulting 3.3-kb deletion encompassing *HIS2* was placed into chromosome *VI* by two-step gene replacement. All deletions described above were confirmed by Southern analysis.

TABLE 2

Recombination at the *HIS2* locus

Diploid	Genotype	Location	Total tetrads	Gene conversions 3:1	1:3	4:0	0:4	G.C.[a] (%)	Associated crossing over[b] (%)
RM101[c]	*his2-1* / *HIS2*		203	16	14	0	0	14.8	47.9
RM183	*his2-1* / *HIS2*		497	36	30	2	2	14.1	
Total	*his2-1* / *HIS2*		700	52	44	2	2	14.3	
RM189[c,d]	*his2-NSI* / *HIS2*	+6	326	8	8	0	0	4.9	
RM167[c,d]	*his2-2* / *HIS2*	+290	312	14	11	0	0	8.0	
RM190[c,d]	*his2-BGL* / *HIS2*	+301	330	22	13	0	0	10.6	47.7
RM193[c]	*his2-390* / *HIS2*	+390	302	24	13	1	0	12.6	50.4
RM168[c,d]	*his2-bam* / *HIS2*	+402	336	15	15	0	0	8.9	41.2
RM191[c,d]	*his2-bcl* / *HIS2*	+509	303	12	19	2	0	10.9	
RM169[c,d]	*his2-xho* / *HIS2*	+749	391	25	26	3	1	14.1	42.1
RM192[c,d]	*his2-acc* / *HIS2*	+885	308	23	22	0	1	14.9	38.0
RM170[c,d]	*his2-hpa* / *HIS2*	+947	377	22	20	1	1	11.7	43.6

For a description of the *his2* alleles, see MATERIALS AND METHODS and Figure 2. The percent gene conversion is calculated as the number of tetrads segregating in a nonmendelian fashion divided by the total number of tetrads times 100.

[a] G.C., gene conversion.

[b] Associated crossing over calculated as the events occurring between the outside markers *cly3* and *cdc14*, corrected for incidental crossovers (as in FOGEL *et al.* 1978).

[c] The relevant genotype of the diploids used to measure crossing over was:

$$\frac{cly3 \quad HIS2 \quad cdc14\text{-}1}{CLY3 \; his2 \quad CDC14}.$$

[d] Conversion data taken from MALONE *et al.* (1992).

Moving the *HIS2* gene to chromosome *II*: An 11-kb clone containing the *LYS2* gene was provided in YEp24 by CARL FALCO (DuPont). From this clone we isolated a 1.9-kb *Eco*RI fragment containing the sequences upstream from the *LYS2* gene (FLEIG *et al.* 1986; MORRIS and JINKS-ROBERTSON 1991) which was inserted into pUC18P[o] (Table 1) creating pJL5 (Table 1). The 2.9-kb *Eco*RI fragment containing the *HIS2* gene was digested with S1 to create blunt ends and inserted into the unique *Pst*I site in the *LYS2* gene in pJL5, which had also been digested with S1, creating pJL7 (Table 1). This clone was transformed into the two haploid parents containing the deletion of *HIS2* on chromosome *VI* (described above), and a two step gene replacement was done to obtain the insertion. To move the 5.2-kb *Bgl*II fragment into the *LYS2* region, the 2.7-kb *Hin*dIII fragment located downstream of *LYS2* was cloned into the *Hin*dIII of pBR322 to create pRM94 (Table 1). The 5.2-kb *Bgl*II fragment containing *HIS2* was cloned into the unique *Bgl*II site in the *LYS2* gene in pRM94 to create pRM97 and pRM98 representing the two possible orientations (Table 1 and Figure 6). These 5.2-kb *Bgl*II insertions were placed in the chromosome as described above for the 2.9-kb *Eco*RI insertion. All *HIS2* insertions into the *LYS2* region were verified by Southern analysis. Finally, to introduce a His$^-$ marker into one of the translocated *HIS2* genes on chromosome *II*, a two step gene replacement was done using the 2.9-kb *Eco*RI fragment containing the *his2-xho* mutation. The final diploid strains were: SK3-151 (the 2.9-kb *Eco*RI insertion) and SK3-231 and SK3-232 (each with one of the two orientations of the 5.4-kb *Bgl*II fragment). (See Table 1 and Figure 6.)

RESULTS

Genetic analysis of the *HIS2* gene: To determine the properties of conversion at *HIS2* in our strains, we examined the conversion frequency in two closely related *his2-1/HIS2* diploid strains (Table 2). RM183 contained the original *his2-1* allele (from RM113-4B), and RM101 contained an identical *his2-1* allele (confirmed by sequencing) that was created by transforming the *his2-1* allele into RM182-55C. That is, each diploid contained all of the 8 changes (7 silent plus the one auxotrophic mutation at position +390) found in the original *his2-1* allele (see below). Both diploids display similar conversion frequencies averaging 14.3%; at least one cell in seven has a meiotic recombination event at

TABLE 3

Properties of the *URA3* insertion at *Hin*dIII site (bp −359)

Diploid	Genotype	Total no. of tetrads	Tetrad type			Gene conversions (at *HIS2*)				G.C. (%)[a]
			P	N	T	$3^+:1^-$	$1^+:3^-$	$4^+:0^-$	$0^+:4^-$	
RM175	*his2-1* / *HIS2::URA3*	83	71	0	0	6	6	0	0	14.5
RM173	*his2-1::URA3* / *HIS2::URA3*	232				24	21	1	1	20.3

The *URA3* gene was inserted into the yeast chromosome by one step gene replacement (see MATERIALS AND METHODS). Its insertion does not result in a *His*$^-$ phenotype. Only nonconversion tetrads in the heterozygote were scored for the segregation of *his2* and *URA3*. The failure to observe N or T type tetrads indicates close linkage (<0.7 cM) between the *URA3* insert and the *HIS2* gene, proving that pH21 does contain *HIS2*. This is further supported by the fact that 5 of the 12 conversions of *HIS2* in the RM175 heterozygote were coconversions of *URA3*. P, parental type; N, nonparental type; T, tetratype.

[a] G.C., gene conversion.

HIS2. We find that conversion at *HIS2* occurs with parity, and, similarly to what FOGEL *et al.* (1978) reported for *HIS2*, we found no postmeiotic segregation (pms) events in the 150 tetrads that we tested for pms. By analysis of heterozygous *cdc14/CDC14* and *cly3/CLY3* markers (in several diploids including RM101—see Table 2) the amount of crossing over associated with conversion at *HIS2* was determined. About 43% of the conversions that occur at the *HIS2* locus are associated with crossing over when corrected (FOGEL *et al.* 1978) for incidental crossing over (Table 2). We conclude that the frequency of gene conversion at the *HIS2* locus is substantially higher than the average in yeast (4%), but that the other properties of the conversion event (parity, association with crossing over, and polarity) are typical of most loci in *S. cerevisiae*.

Cloning the *HIS2* gene: To make alterations in the DNA around the *HIS2* gene, we cloned a fragment of DNA which would complement the *his2-1* mutation. The restriction map for this plasmid (pH21), isolated from a YEp24 library, is shown in Figure 1. To verify that the cloned fragment contained the *HIS2* gene, and not an extragenic suppressor, we inserted the *URA3* gene into the *Hin*dIII site (bp −359) in the *Eco*RI 2.9-kb fragment (Figure 1), transformed a *ura3 his2-1* strain with the purified fragment, and obtained a *HIS2::URA3* transformant. The insertion was verified by Southern hybridization (data not shown). The strain with the insertion was then crossed with a *ura3 his2-1* haploid of opposite mating type and the resulting diploid was dissected. The data in Table 3 indicate that the *URA3* gene is closely linked to the *HIS2* gene and verify that the cloned complementing fragment is indeed the *HIS2* gene. The presence of the heterozygous 1.16-kb *URA3* insertion did not reduce the frequency of conversion at the *HIS2* locus (compare Table 3 and Table 2). We observed no crossovers between the inserted *URA3* and the *HIS2* locus; interestingly, 5 of the 12 conversion events at *HIS2* were coconversion events that also converted *URA3*. These data confirm the conclusion that the *URA3* insert was very close to *HIS2*, and that the fragment of DNA cloned was the *HIS2* gene. We also constructed a diploid homozygous for the *URA3* insert. The homozygous insertion did not reduce the frequency of conversion at *HIS2* (Table 3 and below), indicating that the *Hin*dIII site where the *URA3* fragment was placed was not located in a sequence essential for recombination (Figure 1).

To determine where the *HIS2* gene was located in the fragment isolated in pH21, a number of subclones were tested for their ability to complement the *his2-1* mutation. Figure 1 indicates that the *HIS2* gene is located in the *Eco*RI 2.9-kb fragment, presumably to the right (as the map is drawn) of the *Hin*dIII site discussed above. Further analysis of the region using mini-Tn*3* insertions (see below) confirmed this and further delineated the *HIS2* gene to the region between the *Hin*dIII site and the *Pst*I site (see below).

Sequence analysis and *in vitro* mutagenesis of the *HIS2* gene: To obtain a precise location for the *HIS2* gene, we sequenced the 2.9-kb *Eco*RI fragment (Figure 1). The sequence is shown in Figure 3. The predicted open reading frame for *HIS2* extends for 1005 bp and would generate a protein of 38.5 kD. As predicted for a constitutively produced gene product (STRUHL 1985), there is a poly(dA·dT) tract located upstream (positions −167 to −152) of the putative TATA box (at position −95). We find no *GCN4* binding sequence in the promoter region, consistent with our observation that the amount of *HIS2* mRNA does not increase when cells are grown in the absence of histidine (data not shown). A number of mutations in the *HIS2* gene have been made *in vitro* and were used to analyze the polarity of conversion at *HIS2* (MALONE *et al.* 1992). The data indicated that the conversion gradient was high (14.9%) at the 3′ end of the gene and low (4.9%) at the 5′ end of the gene. The locations of the mutations created by the *in vitro* mutagenesis and used in the experiments in this paper are described in MATERIALS AND METHODS and Figure 2.

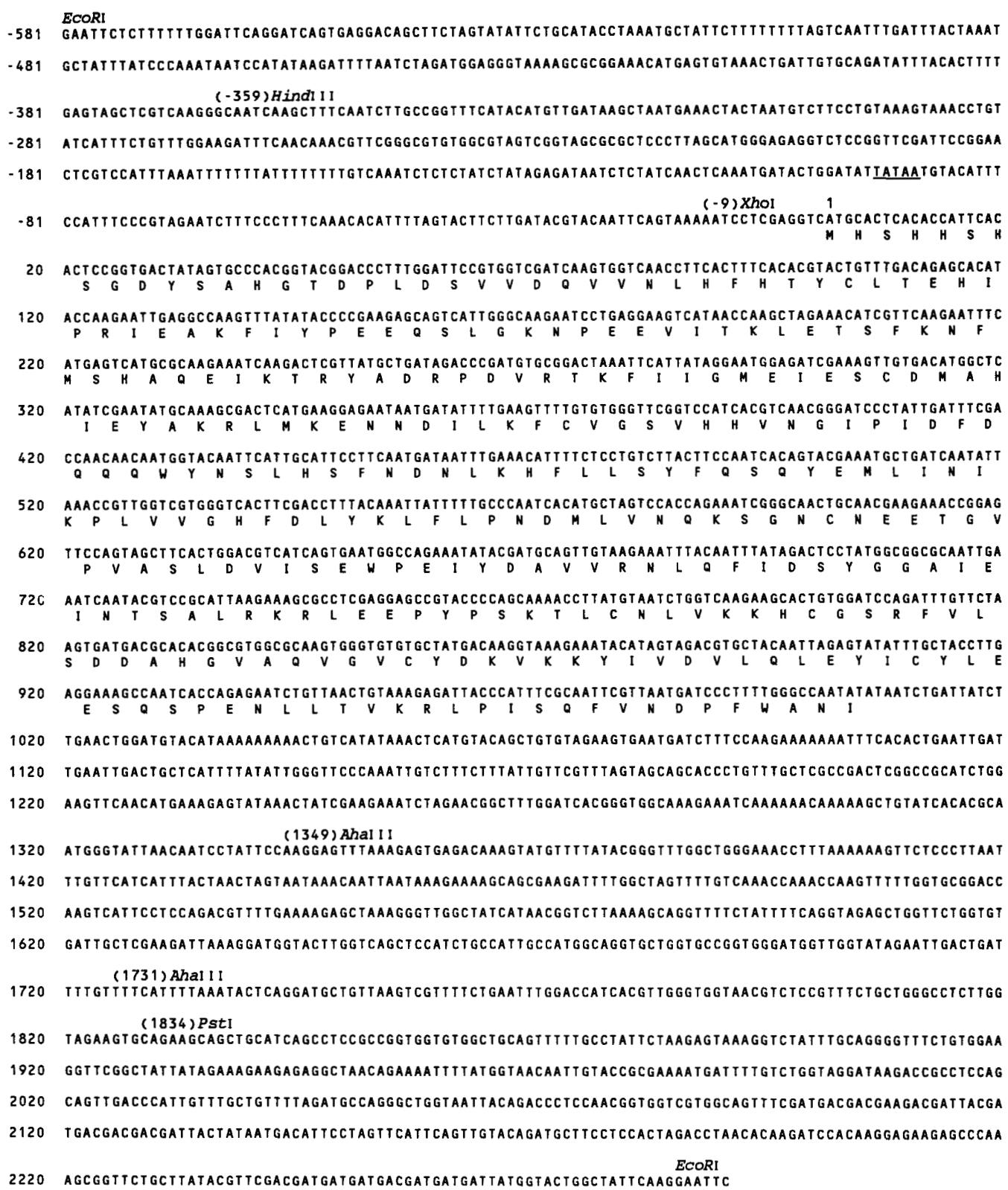

FIGURE 3.—Sequence of the 2.9-kb *Eco*RI fragment containing the *HIS2* gene. The first base pair of the coding region is denoted +1. The coding region extends from +1 to +1005. A poly(dA·dT) tract is located upstream (positions −167 to −152) of the putative TATA box (at position −95) which is underlined. Key restriction sites are shown (see also Figures 1 and 2).

Analysis of the role of the seven silent mutations found in his2-1: The discovery that the allele (*his2-1*) used in much of the analysis of conversion at the *HIS2* locus (see below) contained several mutations other than the one at +390 that conferred histidine auxotrophy (see MATERIALS AND METHODS) was initially disconcert-

ing. BORTS and HABER (1987) reported that the presence of multiple heterologies in a 9-kb interval in a duplication at the *MAT* locus increased gene conversion about seven fold. To determine the possible role of the silent changes at *HIS2* we investigated the conversion frequency of the +390 mutation in a cross where it was the only base change. (We refer to this allele as *his2-390*.) The frequency of gene conversion observed in the *HIS2/his2-390* diploid was 12.6% (Table 2); this was not significantly different from the 14.3% observed for the *HIS2/his2-1* diploid. We also sequenced the *his2-1* allele found in diploid RM173 containing the *URA3* insertion (see Table 3) and found that it contained all seven silent changes as well as the auxotrophic mutation at +390. We therefore must attribute the increase in conversion (from 14.3 to 20.3%) to the insert found in the diploid with the *URA3* insertion, rather than to an alteration in the number of heterozygosities. From the 12.6% conversion frequency of the *his2-390* allele, it appears that the heterozygosities caused by the silent changes do not contribute much, if anything, to the frequency of conversion at *HIS2* (see DISCUSSION and below).

Homozygous Tn*9* and mini-Tn*3* (TUBA) insertions in the HIS2 region: The polarity gradient at *HIS2* suggests that the sequences necessary for high levels of conversion at the *HIS2* gene are located at the downstream end (MALONE *et al.* 1992). To test this prediction, we constructed both insertions and deletions in the *HIS2* region. The general concept of the experimental approach is shown in Figure 4. For the insertion experiments the diploid genotype was *HIS2/his2-1*, which has a basal conversion frequency of 14.3% (Table 2). The locations of the insertions are shown in Figure 5.

Analysis of the conversion frequency in diploids containing homozygous insertions located downstream of *HIS2* confirmed that sequences necessary for the high frequency of gene conversion were located some 700 bp downstream of the 3′ end of the *HIS2* gene (Table 4). First, the 5.5-kb TUBA117 insertion, located at position +1929 (Figure 5), reduced conversion from 14.3 to 6.3%; the 2.6-kb Tn*9*#16 located at position +1925 reduced conversion to 4.2%. Second, the TUBA66 insertion located at position +1701 (to left of the *Pst*I site), reduced conversion to 1.5%. All of these reductions from 14.3% are significant by the G test (SOKAL and ROHLF 1969) at the 99% confidence level (Table 4).

We have rescued and sequenced (see MATERIALS AND METHODS) the putative *his2-1* alleles present in the diploids containing all three insertions above which reduced the frequency of conversion at *HIS2*. Although the data from the *his2-390* allele (which converts at 12.6%) suggest that the seven silent changes could contribute at most 1.7% to the 14.3% conversion observed in *HIS2/his2-1* diploids, we felt it was important to verify that the *his2-1* allele present in the insertion strains was really the original *his2-1* allele. We found that all seven

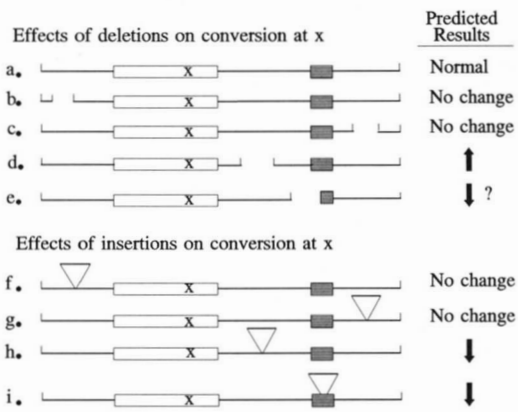

FIGURE 4.—Concept for finding the location of the recombination hotspot for the *HIS2* gene, assuming that the hotspot can be treated as a discrete DNA sequence. (a) Normal chromosome displays normal frequency of conversion. The open box refers to the gene being monitored for recombination, and the X is the mutation being converted. The close vertical lines represent the hotspot sequences. (b) Deletions on the opposite side of the gene should have no effect on conversion since the distance between the hotspot and the gene is unchanged. (c) Likewise, deletions on the far side of the hotspot should have no effect. (d) Deletions that remove DNA between the hotspot and the gene reduce the distance and should increase recombination. (e) Deletions that remove part of the hotspot may reduce conversion. However, since they may also move the remaining sequences closer, such a deletion may have no effect, or even increase recombination. Complete deletions of the hotspot (not shown) should of course decrease conversion. (f) Insertions on the opposite site of the gene should have no effect on conversion. (g) Likewise, insertions on the far side of the hotspot should have no effect. (h) Insertions between the hotspot and the gene should decrease conversion by increasing the distance. (i) Finally, insertions directly in the hotspot should reduce conversion by moving some necessary sequences further away from the gene.

silent changes were present in the *his2-1* allele in the TUBA117 and Tn*9*#16 strains. Thus, the reduction in conversion found in diploids containing these two insertions is attributable to the insertion, rather than to the absence of heterozygosities. The *his2-1* allele in TUBA66 contained only 5 of the seven silent changes; it lacked the two changes at positions +906 and +943. If the absence of these two mismatches were responsible for the entire 1.7% difference found between *his2-1* (14.3%) and *his2-390* (12.6%), it is possible that TUBA66 only decreases conversion to 3.2% rather than 1.5%. Still, it is clear that all three insertions (located further than 700 bp downstream of the coding region) significantly reduce conversion, suggesting that sequences downstream of *HIS2* are important for high levels of gene conversion.

To confirm that the changes in conversion frequency were due to the insertions, rather than anything to do with the *his2-1* allele, we constructed two diploids where the only change in the *HIS2* locus was the *his2-xho* mutation (Figure 2) (conversion frequency 14.1%). We examined two insertions, the TUBA98 insert on the left

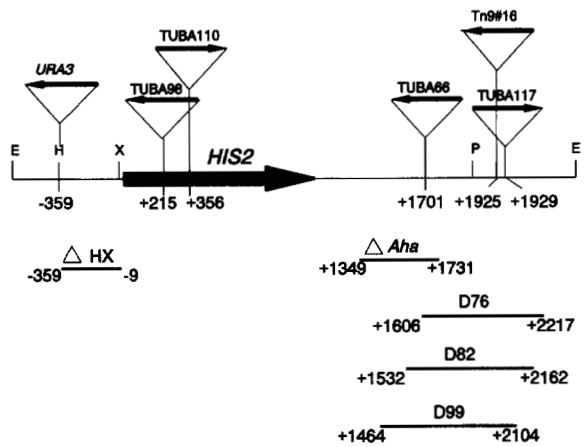

FIGURE 5.—Location of the insertions (shown above the gene) and deletions (shown below the gene) used to analyze recombination at *HIS2*. The *URA3* insertion refers to the 1.16-kb *Hin*dIII fragment; orientation (arrow) is the direction of transcription. The TUBA insertions refer to the 5.5-kb mini-Tn*3* construct (see text); the orientation of the insertion is listed as the direction in which the *lacZ'* fragment would be properly transcribed. The Tn*9* insertion is the 2.6-kb *Escherichia coli* transposon; orientation is the direction of transcription of the *cam*^{r} gene. Numbers immediately below the line refer to the location of the insertion. The end points of the deletions are shown to the left and right of the lines indicating the extent of the deletion. HX, the 350-bp *Hin*dIII-*Xho*I deletion that removes the promoter region of the *HIS2* gene. Aha, the 382-bp *Aha*I deletion downstream of the coding region of *HIS2*. D76, D82 and D99, three different ExoIII/S1 deletions around the *Pst*I site (see MATERIALS AND METHODS). Letters refer to restriction sites as in Figure 1.

side of the gene and the TUBA66 insertion on the right side of the gene. The reduction in conversion for the TUBA66 insertion was 14.1 to 2.2% (compared to 14.3 to 1.5% for *his2-1*) and for the TUBA98 insertion the increase was from 14.1 to 21.6% (compared to 14.3 to 22.6% for *his2-1*). Thus, the insertions, rather than any differences in the number of potential mismatches in different *his2-1* alleles, alter conversion frequency.

Taken together, the overall pattern of the reductions shown by all these insertions suggests that many of the sequences necessary for the high level of conversion at *HIS2* are located to the right of the site of the TUBA66 insertion, and that these sequences are probably not located in one short stretch (see DISCUSSION). Since the TUBA117 insertion at +1929 reduced conversion to 6.3%, it appears that some necessary sequences must lie to the right of this insertion, over 900 bp downstream of the gene being monitored (Figure 5).

The analysis of the insertions located to the left (5' region) of the *HIS2* gene indicate that the DNA 5' to the *HIS2* gene is not necessary for the high frequency of conversion at *HIS2* (Figure 5 and Table 4). The *URA3* insertion located in the *Hin*dIII site at position −359 did not reduce the amount of conversion at *HIS2* (see above); in fact the frequency increased to 20.3% ($0.01 < P < 0.05$). To further test the hypothesis that the 5' end of the gene was not necessary for high levels of conversion, we examined two 5.5-kb TUBA insertions located in the 5' end of the coding region of the *HIS2* gene (Figure 5 and Table 4). Consistent with the *URA3* result, the TUBA98 (at position +215) and TUBA110 (at position +356) insertions did not reduce gene conversion. From these results, one may infer that there are no crucial sequences for the hotspot located at or to the left of +356.

Deletions in the HIS2 region: As described in Figure 4, the analysis of deletions can be somewhat more complicated than that of insertions. If the sequences required for the hotspot are complex, a deletion can remove some of them, but move the remainder closer to the locus being monitored. Thus, the two effects might offset each other. We analyzed several deletions to confirm the results obtained by analysis of insertions (see above). We tested a diploid containing a homozygous deletion of the 382-bp *Aha*III fragment located between bp +1349 and +1731 (Figure 5). This deletion should move the putative sequences necessary for high levels of conversion at *HIS2* closer to the locus. The *Aha*III deletion increased conversion of the *his2-xho* marker from 14.1 to 26.1% ($P < 0.01$) (Table 5).

To further investigate the region to the right of *HIS2* that appeared to be important, three deletions of approximately 600 bp centered around the *Pst*I site (position +1834) were examined in *his2-1/HIS2* diploids. In all crosses the deletion was homozygous (Table 5 and Figure 5). Two deletions (D76, D82) significantly reduced the frequency of conversion, from 14.3 to about 8%, but the third deletion (D99) did not. The effect of the deletions is complicated because each of them moves any remaining distal hotspot sequences closer to the *HIS2* locus (see Figure 4); however, the data indicate that there *are* important sequences located between the endpoints of D99 (+2104) and D76 (+2217) (see DISCUSSION).

Moving the *HIS2* gene to chromosome *II*: The data from the insertions and deletions clearly indicated that necessary sequences for high levels of conversion at the *HIS2* gene were located several hundred base pairs downstream of the gene. To examine the extent of the DNA necessary for the recombination hotspot at *HIS2* at its normal location on chromosome *VI*, DNA fragments containing *HIS2* were inserted adjacent to the *LYS2* gene on chromosome *II* (see Figure 6). If the DNA present moved to chromosome *II* was necessary and sufficient for the high levels of conversion, conversion should occur at the same frequency on II as on *VI*. All analysis was done in diploids homozygous for the insertion and deleted for the *HIS2* gene at its normal location on chromosome *VI*. Thus, ectopic recombination (PETES *et al.* 1991) could not contribute to conversion at *HIS2*. Two fragments were moved to chromosome *II*: the 2.9-kb *Eco*RI fragment and the 5.2-kb *Bgl*II fragment (containing 1283 and 2.2 kb, respectively, downstream of the *HIS2* stop codon; see Figures 1 and 6). The data in

TABLE 4

Effects of insertions on gene conversion at *HIS2*

Strain	Relevant genotype	Type of tetrads				Total tetrads	G.C.[a] (%)	$\frac{3^+:1^-}{1^+:3^-}$
		$3^+:1^-$	$1^-:3^+$	$4^+:0^-$	$0^+:4^-$			
Insertions to right								
SK2-61	*his2-1::TUBA66* / *HIS2::TUBA66*	4	1	0	0	329	1.5**	4.0
RM187	*his2-xho::TUBA66* / *HIS2::TUBA66*	5	2	0	0	316	2.2**	2.5
RM174	*his2-1::Tn9#16* / *HIS2::Tn9#16*	3	3	0	0	143	4.2**	1.0
SK2-42	*his2-1::TUBA117* / *HIS2::TUBA117*	10	6	0	0	253	6.3**	1.7
Insertions to left								
RM173	*his2-1::URA3* / *HIS2::URA3*	24	21	1	1	232	20.3*	1.1
SK2-91	*his2-1::TUBA98* / *his2::TUBA98*	33	20	0	0	234	22.6**	1.7
RM188	*his2-xho::TUBA98* / *his2::TUBA98*	24	43	1	3	328	21.6**	0.56
SK2-111	*his2-1::TUBA110* / *his2::TUBA110*	20	18	0	0	278	13.7	1.1

The conversion frequency for the *his2-1* allele in strains containing no insertions is 14.3% (Table 2); the conversion frequency for the *his2-xho* allele in strains without insertions is 14.1% (Table 2). Insertions "to right" or "to left" are defined by the orientation of standard map (Figure 1). The insertions are described in Figure 5. The percent gene conversion is calculated as the number of tetrads segregating in a nonmendelian fashion divided by the total tetrads times 100. The ratio of $3^+:1^-$ to $1^+:3^-$ tetrads represents the parity of gene conversion. The *TUBA98* and *TUBA110* insertions disrupt the *HIS2* gene, resulting in a His^- phenotype. To monitor conversion in RM188, SK2-91 and SK2-111 diploids standard allelism tests were done on all tetrads to determine whether the *his2-1* allele was present (MALONE 1983). $^*P < 0.05$ that the difference from the strain without insertions is due to random chance; $^{**}P < 0.01$ that the difference from the strain without insertions is due to random chance.

[a] G.C., gene conversion.

TABLE 5

Effects of deletions on gene conversion at *HIS2*

Diploid	Relevant genotype	Type of tetrads				Total tetrads	Percent conversion frequency	$\frac{3^+:1^-}{1^+:3^-}$
		$3^+:1^-$	$1^+:3^-$	$4^+:0^-$	$0^+:4^-$			
RM102	*HIS2-ΔHX* / *his2-hpa-ΔHX*	18	25	0	0	214	20.1 (11.7)**	0.7
LL2-34	*HIS2-ΔAha* / *his2-xho-ΔAha*	17	29	0	1	180	26.1 (14.1)**	0.6
JL4-15	*HIS2-D76* / *his2-1-D76*	10	8	0	0	210	8.6 (14.3)*	1.2
JL4-9	*HIS2-D82* / *his2-1-D82*	8	12	0	0	249	8.0 (14.3)**	0.7
JL4-7	*HIS2-D99* / *his2-1-D99*	16	12	2	2	245	13.1 (14.3)	1.3

Values in parentheses next to conversion frequency indicate the basic level of conversion observed in strains without the deletion (see Table 2). The location of the deletions is described in Figure 5. The $3^+:1^-/1^+:3^-$ ratio represents the parity of gene conversion. $^*P < 0.05$ that the difference between the deletion strain and a nondeleted strain is due to random chance by *G* test; $^{**}P < 0.01$ that the difference is due to random chance.

Table 6 indicate that the *HIS2* gene no longer displays a high frequency of recombination, nor is there any effect on recombination at the *LYS2* locus (see DISCUSSION).

DISCUSSION

For the two cases in *S. cerevisiae* in which meiotic recombination hotspots have been extensively studied by both molecular and genetic approaches (*i.e.*, *ARG4* and *HIS4*), the sequences necessary for the high frequency of gene conversion appear to be located in the promoter region within a few hundred bases of the 5′ end of the coding region (NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1991; WHITE *et al.* 1991). In fact, Rap1 binding sites or poly (dA·dT) sequences that have

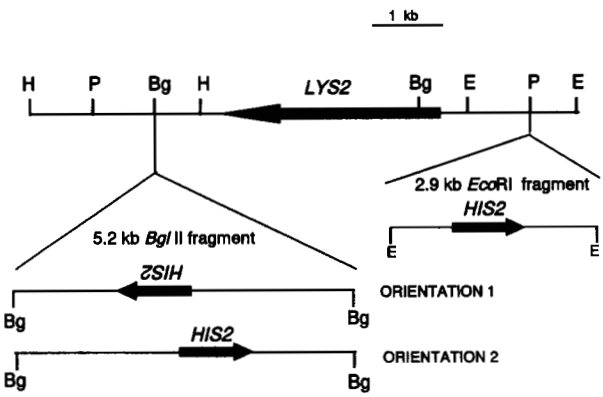

FIGURE 6.—Fragments used to move *HIS2* to chromosome *II* near the *LYS2* gene. Letters refer to restriction sites as in Figure 1. Two orientations of the larger *Bgl*II fragment were examined and are indicated as "1" and "2." (See text for details.)

a functional role in the promoters of these genes appear to contribute to high levels of conversion at these two loci. However, transcription *per se* does not appear to be required for high levels of gene conversion at either *ARG4* or *HIS4* (SCHULTES and SZOSTAK 1991; WHITE *et al.* 1991). The situation appears to be different for the *HIS2* gene; a summary of the effects of deletions and insertions in the vicinity of *HIS2* is shown in Figure 7. The overall pattern is consistent with the location of necessary sequences located several hundred base pairs downstream of the *HIS2* gene. We note that all major alterations of sequences confer a degree of uncertainty: not only can sequences present be deleted or moved further away, but new junction sequences can be formed which may themselves affect recombination. Our arguments about which sequences are important for the initiation of recombination at *HIS2* are based on the overall effect of *all* the deletions and insertions, as well as the movement of the gene to another chromosome.

Analysis of alterations at the 5′ end of *HIS2*: Gene conversion at *HIS2* is highest toward the 3′ end of the gene and lower at the 5′ end (MALONE *et al.* 1992); in the simplest situation, sequences located at the 5′ end of the gene should not be required for high levels of conversion. While two different types of insertions and a deletion at the 5′ end *do not* decrease conversion, three 5′ alterations significantly increase recombination (Figure 7). One interpretation of these observations is that sequences 5′ to the coding region inhibit conversion; deleting them, or moving them further away, increases conversion. Looking at all the evidence together, there is no indication that sequences located 5′ to the coding region are necessary for conversion at *HIS2*.

Although two insertions (TUBA110 and TUBA98) at the 5′ end did not reduce conversion, their effect on conversion was different (13.7 *vs.* 22.6%). One possible explanation is that TUBA110 and TUBA98 are inserted in opposite orientations. If *HIS2* promoter sequences inhibit recombination (see above), perhaps the two different orientations differentially reduce transcription or activity at the promoter. Consistent with this interpretation, the 350 bp *Hin*dIII-*Xho*I deletion of the promoter region also increases recombination from 11.7% (for the *his2-hpa* allele) to 20.1% (MALONE *et al.* 1992) (Table 2). The deletion of the promoter region removes a large tract (bp −174 to −151) of poly(dA·dT); thus this particular motif does not appear to be required for the high frequency of recombination exhibited at *HIS2*. An alternative possibility for the difference between the effect of TUBA98 and TUBA110 is that they create different junction sequences that affect recombination differently. Whatever the cause for the difference, we emphasize that neither alteration *decreases* conversion.

Analysis of the region downstream of *HIS2*: All of the alterations located downstream of *HIS2* affect recombination as predicted if necessary sequences for the hotspot were located approximately 700 bp 3′ to the end of the coding region (Figure 7). The *Aha*III deletion, which moves these proposed hotspot sequences closer, increases conversion from 14.1 to 26.1%. Consistent with this, all three insertions located downstream of bp +1700 lower conversion. This is predicted if sequences to the right of the insertions are necessary for high levels of conversion. The pattern of reduction [TUBA66 (1.5–3.2%) > Tn*9*#16 (4.2%) > TUBA117 (6.3%)] is consistent with the hotspot being complex; an insertion located further to the right leaves some of the sequences necessary for high levels of conversion close to the *HIS2* locus.

Deletions in this 3′ region are consistent with this interpretation. The ExoIII deletions around the *Pst*I site (+1834) remove some of the sequences defined by the insertions as necessary for recombination (Figure 7). Two of the deletions (D76 and D82) reduce conversion, but only to 8%. We interpret this as an indication that there are some necessary sequences remaining, and that these are simultaneously moved closer to the *HIS2* gene. In the case of D99 (which removes the smallest portion of the proposed necessary sequences and the largest amount of DNA between them and *HIS2*), we propose that the two effects offset each other leading to no significant change (14.3 *vs.* 13.1%) in conversion frequency (see also Figure 4). The analysis of deletions and insertions downstream of *HIS2* suggests that some sequences necessary for high level conversion are located between bp +1701 (the TUBA66 insertion) and bp +2217 (the right end of deletion D76). It is clear from the data that additional sequences downstream of +2217 must also be required. The apparently additive property of the necessary sequences is unusual; by additive, we mean that the greater the extent of necessary sequences close to the *HIS2* gene, the higher the conversion frequency. The available data are consistent with the notion that the more "hotspot sequences" available, the higher the amount of conversion. While other types

TABLE 6

Gene conversion of fragments containing the *HIS2* locus moved to chromosome *II*

Diploid	Relevant genotype on chromosome *II*	Relevant genotype on chromosome *VI*	Conversion frequency on *VI HIS2*	Conversion frequency on *II HIS2*	Conversion frequency on *II LYS2*
Controls					
RM196		*his2-xho* / *HIS2*	14.1% (55/391)	N.R.	N.R.
Eight strains[a]	*lys2-1* / *LYS2*		N.R.	N.R.	0.4% (5/1,309)
The *Eco*RI 2.9-kb fragment					
SK3-151	*lys2-1::HIS2* / *LYS2::his2-xho*	Δ(*his2*) / Δ(*his2*)	N.R.	2.0%** (3/153)	<0.7% (0/153)
The *Bgl*II 5.2-kb fragment					
SK3-231	*lys2-1::HIS2(2)* / *LYS2::his2-xho(2)*	Δ(*his2*) / Δ(*his2*)	N.R.	1.2%** (3/243)	0.4% (1/243)
SK3-232	*lys2-1::HIS2(2)* / *LYS2::his2-xho(2)*	Δ(*his2*) / Δ(*his2*)	N.R.	0.4%** (1/251)	<0.4% (0/251)

The amount of conversion when the *HIS2* locus was moved to chromosome *II* is compared with the amount of conversion that normally occurs at chromosome *VI*. Values in parentheses indicate the actual ratio of conversion to total tetrads analyzed. Δ(*his2*) represents the deletion of a 3.3-kb *Eco*RI fragment containing *HIS2* from its normal location on *VI* (see text). N.R., not relevant. The numbers 1 or 2 in parentheses in the genotype of diploids SK3-231 and SK3-232 refer to the two possible orientations of the 5.2-kb *Bgl*II fragment (see Figure 8). $*P < 0.05$; $**P < 0.01$ by *G* test.

[a] Because the frequency of conversion of the *lys2-1* allele is low, data were pooled from eight closely related diploids.

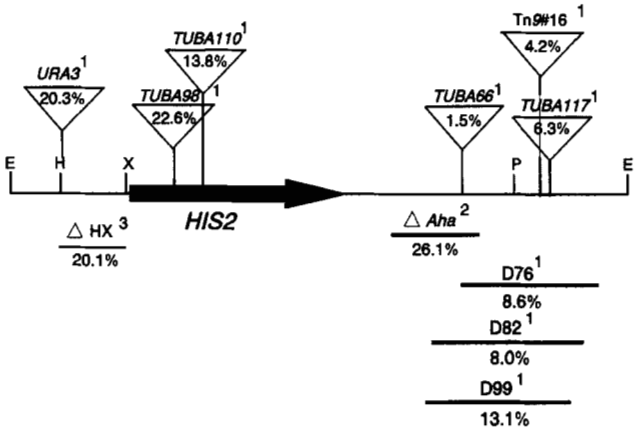

FIGURE 7.—Summary of the effects on conversion of all insertions and deletions analyzed. The numbers within the triangles below each insertion name indicate the amount of gene conversion observed in the diploid homozygous for that insertion. The numbers below the lines representing the extent of the deletion indicate the amount of conversion observed in the diploid containing the homozygous deletion. The allele used to monitor conversion was the *his2-1* allele (see text for details) and is indicated by the superscript 1 (basal conversion level = 14.3%) except for: (1) the *Aha* deletion was analyzed using the *his2-hpa* allele (indicated by the superscript 2; basal level = 11.7% conversion), and (2) The *Hin*dIII-*Xho*I deletion was analyzed using the *his2-xho* allele (indicated by the superscript 3; basal level = 14.1% conversion). Letters refer to restriction sites as in Figure 1.

of *cis*-acting sites such as promoters can extend over hundreds or even thousands of bases, deletion or inactivation of their component parts often result in a major (several log) effect (*e.g.*, alteration of a TATA box).

Recently, STAPLETON and PETES (1991) examined the effect on recombination of a mini-Tn*3* transposon (inserted into the region between *LEU2* and *CEN3*) that is related to the TUBA insertions (SIEFERT *et al.* 1986) used in these experiments. The mini-Tn*3* element contained the *URA3* gene and the *amp*^{r} gene but lacked the 3.2-kb *lacZ*′ fragment in the TUBA element. The transposon used by STAPLETON and PETES increased both crossing over and conversion in the *LEU2-CEN3* interval; they attributed the increase in recombination to a hotspot near the 5′ end of the *amp*^{r} gene. Their results are very different from the reductions in conversion that we observe using the TUBA insertions above; the TUBA insertions clearly are not acting as "hotspots" for recombination when located downstream of *HIS2*. Considering all the data in this paper together, we suggest that the simplest hypothesis is that the TUBA insertions do not act as hotspots. The difference between our observations and those of STAPLETON and PETES may be due to the presence of the *lacZ*′ DNA in the insertions we used, or that we are examining a different region of the genome.

We have examined the sequences between +1700 and +2284 to determine if they contain motifs reported to be important in recombination in yeast or in other organisms [*e.g.*, Z-DNA (PROUDFOOT and MANIATIS 1980); mouse MHC hotspot (KOBORI *et al.* 1986); Chi sites (MALONE *et al.* 1978)]. We have not found such sequences. Likewise we have found no significant homology to the sequences reported to be necessary for high level conversion at *ARG4*. Repeated sequences have been proposed to act as recombination hotspots in a

number of organisms, including yeast (*e.g.*, TRECO and ARNHEIM 1986). Although none of the published putative hotspot repeat sequences appear in the region, we do note the presence of a series of 5′-GAPy-3′ repeats in the 290-bp region between +1980 and +2270. There are 21 repeats of this motif, including a span of 36 bp that contains 10 repeats between bp +2097 and +2132, and a run of 8 repeats between bp +2241 and +2264. We are currently determining whether these repeats are important for meiotic gene conversion at *HIS2*.

We have attempted to determine how much DNA was required for the *HIS2* hotspot by moving two fragments of DNA containing the *HIS2* gene to another chromosome. In both experiments, the *HIS2* gene had a low level of conversion, even though in one case more than 2.2 kb of downstream DNA was moved. There are several possible explanations for the low frequency of conversion at *HIS2* observed in these two experiments. First, it is possible that all of the sequences necessary for the hotspot are not present in the DNA moved to chromosome *II*. If so, this would indicate that the hotspot "site" is spread over several kilobases of DNA. Second, all the necessary sequences for high levels of conversion may be present, but the *LYS2* region of chromosome *II* may contain specific sequences that constitute a "cold spot" for recombination. For this hypothesis to be true, one would have to suppose that such cold spots are dominant to hotspots. A third possibility is that hotspots, at least in the form of discrete, defined DNA sequences, do not exist. Rather, hotspots occur only because of chromosome context or higher order structure. Of course, the DNA sequences present ultimately determine this proposed higher level structure, but not in the same way that origins of replication consist of specific sequences where the replication complex acts, or that promoters consist of specific sequences that allow RNA polymerase to bind. It is possible to move an origin or a promoter to other locations where they apparently can function normally (*e.g.*, ZAKIAN and SCOTT 1982); it may not be generally possible to do so for recombination with limited amounts (a few kilobases) of DNA. Of course, if one moves a large enough fragment of DNA, the higher order structure would presumably be able to form at the new location. Consistent with this idea are the data of ROSS *et al.* (1992); a 12.5-kb fragment containing *ARG4* placed in an artificial chromosome retained between 55% to 75% of its normal conversion level. Finally, it is possible to propose a hybrid hypothesis: recombination hotspots may indeed require a number of discrete component sequences, but these sequences may function only when present in the right chromosomal context as has been found in *S. pombe* (PONTICELLI and SMITH 1992). From analysis of double strand breaks (dsb's) in the same sequence located at different positions in the genome, GOYON and LICHTEN (1993) came to the conclusion that DNA sequence alone does not determine whether a dsb will occur. If dsb's act to initiate recombination in meiosis, their observations appear consistent with the last hypothesis. We are currently attempting to distinguish among these possibilities.

The role of mismatches in conversion: The lack of any significant effect on gene conversion due to mismatches caused by the seven silent mutations present in the *his2-1* allele is interesting. Perhaps the difference between our results and those of BORTS and HABER (1987) is due to the fact that their heterologies resulted from restriction site ablations, whereas the silent mutations in the *his2-1* allele are single base changes. Alternatively, the decrease in crossing over and the sevenfold increase in conversion observed by BORTS and HABER (1987) when heterologies were present may have been due to the *MAT* duplication bracketing the alterations. The several kilobases of homologous *MAT* DNA on either side of the heterozygosities might affect the events being measured. Consistent with this latter hypothesis is the observation that multiple restriction site ablations between the *LEU2* gene and the centromere (also on chromosome *III*) did not appear to increase or decrease the normal frequency of recombination (SYMINGTON and PETES 1988).

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