Genetic Evidence That the Meiotic Recombination Hotspot at the *HIS4* Locus of *Saccharomyces cerevisiae* Does Not Represent a Site for a Symmetrically Processed Double-Strand Break

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Manuscript received July 15, 1992
Accepted for publication January 5, 1993

ABSTRACT

In the yeast *Saccharomyces cerevisiae*, the binding of the Rap1 protein to a site located between the 5′ end of the *HIS4* gene and the 3′ end of *BIK1* stimulates meiotic recombination at both flanking loci. By using strains that contain mutations located in *HIS4* and *BIK1*, we found that most recombination events stimulated by the binding of Rap1 involve *HIS4* or *BIK1*, rather than bidirectional events including both loci. The patterns of aberrant segregation indicate that most of the Rap1-stimulated recombination events do not represent the symmetric processing of a double-strand DNA break located at the Rap1-binding site.

DURING meiosis in *Saccharomyces cerevisiae*, heterozygous alleles *A* and *a* usually segregate with a Mendelian pattern of 2*A*:2*a* spores. Non-Mendelian or aberrant segregation patterns occur with a frequency of about 5% for the average gene in yeast (reviewed in PETES, MALONE and SYMINGTON 1991). For most mutant alleles, the most common type of aberrant segregation pattern is gene conversion. Conversion events represent the nonreciprocal transfer of information from one allele to the other, resulting in tetrads containing either 3*A*:1*a* or 1*A*:3*a* spores. The second class of aberrant segregants, postmeiotic segregation (PMS) events, are usually much less common than gene conversion events (FOGEL, MORTIMER and LUSNAK 1981; PETES, MALONE and SYMINGTON 1991). PMS events are detected as sectored colonies derived from a single spore in which the two alleles segregate (ESPOSITO 1971). In general, tetrads with PMS events have a single sectored colony (FOGEL, MORTIMER and LUSNAK 1981; PETES, MALONE and SYMINGTON 1991). PMS tetrads are usually described using the nomenclature developed for eight-spored fungi. Thus, tetrads with the segregation pattern 2*A*:1*a*:1 sectored *A*/*a* spore colony are called "5*A*:3*a*" tetrads and those with the pattern 1*A*:2*a*:1 sectored *A*/*a* spore colony are called "3*A*:5*a*" tetrads. Tetrads with the pattern 1*A*:1*a*:2 *A*/*a* spore colonies are called "aberrant 4:4" tetrads. By this nomenclature, gene conversion events represent 6:2 or 2:6 events and normal Mendelian segregations are 4:4 events. This nomenclature will be used to describe aberrant segregation events below, where aberrant segregation is the sum of gene conversion and PMS.

Both gene conversion and PMS events are thought to be mechanistically related to meiotic crossovers. One indication of a relationship is that conversion or PMS events involving a genetic marker are nonrandomly associated with crossovers of flanking sequences (FOGEL and HURST 1967). PMS events are thought to reflect unrepaired mismatches in heteroduplexes containing one strand of wild type and one strand of mutant information. Genetic studies suggest that, in *S. cerevisiae*, most gene conversion events reflect the repair of mismatches formed in heteroduplexes involving wild-type and mutant information (FOGEL, MORTIMER and LUSNAK 1981; NAG, WHITE and PETES 1989). Thus, the observation that most alleles in yeast have much more gene conversion than PMS indicates that most mismatches are efficiently repaired in meiosis. Exceptions to this generalization are C/C mismatches (WHITE, LUSNAK and FOGEL 1985; LICHTEN *et al.* 1990; DETLOFF, SIEBER and PETES 1991), certain small deletions (WHITE, LUSNAK and FOGEL 1985), and small palindromic insertions (NAG, WHITE and PETES 1989).

Our study concerns meiotic recombination at the *HIS4* locus. In our genetic background, this locus has a very high frequency of aberrant segregation; up to 50% of unselected tetrads show non-Mendelian segregation (NAG, WHITE and PETES 1989; DETLOFF, SIEBER and PETES 1991). As with a number of genes studied in yeast (FOGEL, MORTIMER and LUSNAK 1981; PETES, MALONE and SYMINGTON 1991), the *HIS4* locus has a gradient of gene conversion for low PMS alleles along the gene. Alleles at the 5′ end have a frequency of gene conversion that is about 2.5-fold higher than alleles at the 3′ end (DETLOFF, WHITE and PETES 1992). In addition, the frequency of gene conversion at *BIK1*, which is directly upstream of *HIS4*, is significantly lower than the frequency at the

5′ end of *HIS4*, demonstrating that the peak of the conversion gradient is between the 5′ end of *HIS4* and the 3′ end of *BIK1* (DETLOFF, WHITE and PETES 1992).

We have recently found that deletions in the region between the 5′ end of *HIS4* and the 3′ end of *BIK1* lower the frequency of recombination of markers at the 5′ end of *HIS4* and markers in *BIK1* (DETLOFF, WHITE and PETES 1992). This region has the upstream activating sequences (UAS) for *HIS4* and contains the binding sites for the transcription-activating proteins Bas1, Bas2, Rap1 and Gcn4. Although this upstream region contains one or more sites that stimulate recombination in two directions, it is not clear from these studies whether individual events are bidirectional. We also showed that one important factor for the activity of the hotspot is the binding of the Rap1 protein to its binding site within the UAS (WHITE *et al.* 1991). Although most of the alterations that reduce recombination also reduce the level of transcription, we found that deletion of the TATAA sequence required for efficient transcription of *HIS4* had no effect on *HIS4* recombination (WHITE *et al.* 1992).

Other features of recombination that have been investigated at the *HIS4* locus are the specificity of strand transfer during heteroduplex formation and the direction of mismatch repair within the heteroduplex. As described above, genetic studies in yeast suggest that most heteroduplexes are formed by the asymmetric transfer of a single strand of DNA from one allele to the other. At the *HIS4* (NAG and PETES 1990) and *ARG4* (LICHTEN *et al.* 1990) loci, the nontranscribed strand is preferentially transferred in the formation of these heteroduplexes. Mismatches within heteroduplex DNA can be repaired in two different ways. If the mismatch is excised from the donor strand in the heteroduplex and the resulting gap is repaired using the recipient strand, an aberrant segregation event is not detected (restoration-type repair). Alternatively, if the mismatch is removed from the recipient strand and the gap is repaired using the donor strand, a gene conversion event occurs. In a previous study, we found that mutant alleles at the 5′ end of *HIS4* had approximately the same frequency of aberrant segregation whether the mutations resulted in well repaired or poorly repaired mismatches (DETLOFF, WHITE and PETES 1992); however, at the 3′ end of *HIS4* and in *BIK1*, mutant alleles with poorly repaired mismatches (high PMS alleles) had a significantly higher level of aberrant segregation than that observed for a well repaired allele at the same position in the gene. The simplest interpretation of this result is that most well repaired mismatches at the 5′ end of *HIS4* undergo conversion-type repair, whereas those located at the 3′ end of *HIS4* and in *BIK1* undergo both conversion- and restoration-type repair.

As mentioned above, mutations within the Rap1-binding site reduce the frequency of meiotic recombination at *HIS4*, and a 150-base pair (bp) deletion that removes the Rap1-binding site reduces the frequency of recombination at *HIS4* and *BIK1*. For high PMS alleles, most aberrant segregants (about three-fourths) are PMS events, and the remainder are gene conversions. The deletion of the Rap1-binding site reduces the level of aberrant segregation for a high PMS allele at the 5′ end of *HIS4* by twofold (WHITE *et al.* 1991). The frequency of aberrant segregation for a high PMS allele is likely to primarily reflect the level of heteroduplex formation, although the possibility that the conversion events that occur for high PMS alleles represent some mechanism other than mismatch repair within a heteroduplex cannot be excluded. Thus, the above result suggests that approximately half of the heteroduplexes formed at the 5′ end of *HIS4* are Rap1-dependent. In this study, we show that about half of the heteroduplexes formed at *BIK1* are also Rap1-dependent. In addition, we show that most recombination events stimulated by Rap1 involve *HIS4* or *BIK1*, rather than bidirectional involvement of both sites. These results indicate that most of the heteroduplexes stimulated by Rap1 are not a consequence of a double-strand break of DNA at the Rap1 site that is processed symmetrically toward the *HIS4* and *BIK1* genes. Other mechanisms consistent with our observations will be discussed below.

MATERIALS AND METHODS

Plasmids: The plasmid pSP3 was used to make the *bik1-IR16* mutation and was constructed using standard cloning techniques. The plasmid pCIG-17 contains a *Bam*HI fragment of yeast chromosome *III* (inserted in the *Bam*HI site of YIp5) that is identical to pCIG (NEWLON *et al.* 1986; RAD *et al.* 1991), except that the *Sal*I site located in the *HIS4* gene has been "filled in," creating a 4-bp insertion. A *Xho*I fragment containing the 3′ end of *BIK1* and the 5′ end of *HIS4* was excised from pCIG-17 (see Figure 1), and inserted into the *Sal*I site of B142. The plasmid B142 is identical to YIp5 (GUTHRIE and FINK 1991) except for an approximately 250-bp deletion removing the *Pvu*II site. The palindromic oligonucleotide IR16 (provided by D. NAG) was inserted at the *BIK1 Sal*I site and the correct sequence confirmed by DNA sequencing using the Sequenase system as described by the manufacturer (U. S. Biochemical). The oligonucleotide IR16 has the sequence 5′-TCGAGTACTGTATG-TGGATCCACATACAGTAC-3′.

The plasmid pMW35 was used in the construction of yeast strain SPY202. This plasmid was made by excising the *Sau*3AI fragment of pCB600 (DEVLIN *et al.* 1991), and inserting this fragment into the *Bam*HI site of the vector B142. The *Sau*3AI fragment of pCB600 contains the *his4-51* mutation that changes 4 bp in the Rap1-binding site at the 5′ end of *HIS4* (DEVLIN *et al.* 1991).

Yeast strains: All strains used were derived from the haploid strains AS13 (*MAT***a** *leu2 ura3 ade6*) and AS4 (*MATα trp1 arg4 tyr7 ade6 ura3*) (STAPLETON and PETES 1991), except for the *bik1* tester strains. SPY4 was derived

from strain PD100 (DETLOFF and PETES 1992), an AS13 derivative with two palindromic oligonucleotides inserted in the *HIS4* gene. The 18-bp oligonucleotide IR9 (NAG and PETES 1991) is inserted at the *HIS4 SalI* site located at +467 relative to the initiating codon, and the 27-bp oligonucleotide 3133 (DETLOFF, WHITE and PETES 1992) is inserted at the *XbaI* site located at +2327. The *bik1-IR16* mutation was inserted into PD100 using pSP3 with the two-step transplacement technique (SCHERER and DAVIS 1979; GUTHRIE and FINK 1991), yielding the strain SPY4. The *bik1-IR16* mutation creates a new *Bam*HI site; the location of this site in the SPY4 genome was confirmed by performing a Southern blot using the method supplied with Hybond-N (Amersham). The strain SPY11 was derived from SPY4 by replacing the wild-type Rap1-binding site with the mutation *his4-51* by using the plasmid pMW35 and the two-step transplacement as described above. The presence of this mutation was confirmed by performing a Southern blot of DNA isolated from SPY11, since the *his4-51* substitution creates a new *Sac*I site at the position of the mutation. The diploid SPY200 was constructed by mating SPY4 with AS4, and SPY202 was made by mating SPY11 to MW30 (an AS4-derived haploid with the *his4-51* mutation inserted upstream of a wild-type *HIS4* gene) (WHITE *et al.* 1991).

Two other diploid strains (DNY11 and MW118) derived from AS4 and AS13 were used to examine the specificity of strand transfer. The strain DNY11 (NAG, WHITE and PETES 1989) is heterozygous for the *his4-lop* mutation (a palindromic insertion) and has wild-type sequences upstream of *HIS4*. The strain MW118 (WHITE *et al.* 1991) is also heterozygous for *his4-lop* and, in addition, is homozygous for the *his4-51* mutation (mutant Rap1-binding site).

Other strains in this study were used in mating assays that confirmed the presence of specific markers. The strains used to check for the presence of the *his4-IR9* mutation were PD21 (*MAT***a** *leu2 ade6 ura3 his4-Δ29*) and PD68 (*MATα trp1 arg4 tyr7 ade6 ura3 his4-Δ29*). The strains used to check for the presence of the *his4-3133* mutation were PD22 (*MAT***a** *leu2 ade6 ura3 his4-712*) and PD25 (*MATα trp1 arg4 tyr7 ade6 ura3 his4-713*). All of the *his4* tester strains were provided by P. DETLOFF (DETLOFF and PETES 1992).

The strains used to check for the presence of the *bik1-IR16* mutation were L1545 (*MAT***a** *lys9 his4-Δ453*) and L1546 (*MATα lys9 his4-Δ453*) provided by J. TRUEHEART. The *his4-Δ453* deletion removes part of the *HIS4* gene, all of the *BIK1* gene, and part of the *FUS1* gene, and thus represents a *bik1* null mutation (TRUEHEART, BOEKE and FINK 1987).

Genetic analysis: Standard procedures and media were used (GUTHRIE and FINK 1991). Diploids were sporulated on plates containing 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 6 μg/ml adenine and 2% agar at 18° for 4–5 days. Cells sporulated under these conditions have a level of conversion at *HIS4* that is about twofold higher than that observed after sporulation at 25° (NAG, WHITE and PETES 1989). The efficiency of sporulation at 18° is about 10–25%, compared to the 50% value observed at 25°. Tetrads were dissected onto YPD plates (1% yeast extract, 2% Bacto-peptone (Difco), 2% dextrose and 2% agar), and allowed to grow at 30° for 3–4 days. The spore colonies were replica-plated onto appropriate omission media for subsequent analysis.

The *HIS4* gene encodes a multifunctional protein, and has three complementation groups termed *HIS4A*, *HIS4B*, and *HIS4C* (JONES and FINK 1982). Since *his4-IR9* is an in-frame mutation in *HIS4A* and *his4-3133* is a mutation in *HIS4C*, it is possible to distinguish the mutations by complementation analysis in which the spores are mated to **a** and α tester strains that are either either *his4A his4B HIS4C* (PD21 and PD68) or *HIS4A HIS4B his4C* (PD22 and PD25). After mating, the spore colonies were replica-plated to medium lacking histidine to determine if the *his4* mutation could be complemented by either of the tester strains (DETLOFF and PETES 1992). Since both of the mutations are present on the same chromosome initially, only a recombination event can result in a spore that has only one mutant allele. Spore colonies on the plates lacking histidine were examined microscopically in order to detect sectored His^+/His^- colonies (nongrowing cells were visible in the His^- part of the sector).

The *bik1* mutation results in a defect in karyogamy following mating (TRUEHEART, BOEKE and FINK 1987). Consequently, cells with a *bik1* mutation form diploids inefficiently when crossed to *bik1* cells of the opposite mating type. To determine which of the spores had the *bik1-IR16* mutation, we mated the spore colonies to the *bik1* tester strains L1545 and L1546 for 2 hr at 25°, and then replica-plated the mated cells to SD minimal medium (0.17% Difco yeast nitrogen base without amino acids or ammonium sulfate, 0.5% ammonium sulfate, 2% dextrose, and 2% agar) supplemented with 0.003% histidine. Since the *bik1* cells mate to some extent, all colonies on the minimal plates were examined microscopically. Since the *bik1-IR16* allele had a high level of postmeiotic segregation, another purpose for the microscopic examination was to detect a small *bik1* sector in a colony that was predominantly *BIK1*. The colony morphology of mated cells that were homozygous for *bik1* was easy to distinguish from mated cells that had one *BIK1* parent, since the homozygotes produced ragged-edged colonies that were clearly different from the smooth-edged colonies produced if one parent was wild type. To further confirm the genotype of the spores, we analyzed many of the spore colonies by polymerase chain reaction (PCR) (see below). The specificity of strand transfer for heteroduplexes involving the *HIS4* locus in strains DNY11 and MW118 was examined as described previously (NAG and PETES 1990).

PCR analysis: To provide a further check on our ability to detect spore colonies that were sectored for the phenotype caused by the *bik1* mutation, we performed PCR analysis on many of the spore colonies; the PCR procedure for yeast colonies was based on methods provided by M. OLSON (Washington University). We used a DNA Thermal Cycler (Perkin-Elmer Cetus). The reaction conditions for the PCR analysis were those provided with the AmpliTaq polymerase from Perkin-Elmer Cetus, with several modifications. To be sure both sides of a potential sector were detected, we used a new sterile toothpick to mix the cells of the spore colony on a YPD plate. A toothpick was streaked through the middle of the patch and the resulting cell sample was suspended in 5 μl of sterile distilled water in a 1.5-ml Eppendorf tube. The samples were heated to 95° for 4 min to inactivate nucleases and proteases, and the remainder of the reaction components was added. The optimized standard buffer contained 3 mM $MgCl_2$. The samples were run through 30 cycles of the following program: 94° for 1 min to denature, 58° for 2 min to anneal, and 72° for 3 min for synthesis. After reactions were completed, the samples were centrifuged in a microcentrifuge for 2 min to pellet cell debris and half of the sample was used for gel electrophoresis in 1% agarose.

Each sample was analyzed with two sets of primers. One set of primers produced a 700-bp PCR product only if the wild-type *BIK1 SalI* site was present (oligonucleotides SEP4 5′GCTATACGTTTGTATCGC3′ and SEP5 5′ACAGGATAGAGTCGACCT3′) and produced no band if the *bik1-*

IR16 allele was present. The other set of primers produced a 1040-bp PCR product only if the *bik1-IR16* allele was present (SEP2 5′ATCCACATACAGTACTCG3′ and MW2714 5′CACACATCGGAGGTGAAT), and produced no band if the wild-type *BIK1* allele was present. A colony that had a sectored *BIK1/bik1* genotype would produce both bands. Every set of reactions was run with three control colonies that were known to be wild type, completely mutant, and sectored for *BIK1*. In general (more than 90% of the time), there was very good agreement between the microscopic scoring and PCR scoring techniques. When the two assays gave different results, the PCR data were used because of their accuracy in identifying very small sectors.

RESULTS

As described in the Introduction, we previously identified a region located between the *HIS4* and *BIK1* genes that stimulated recombination of markers located in the *HIS4* and *BIK1* genes (WHITE *et al.* 1991; DETLOFF, WHITE and PETES 1992). In addition, we found that mutations in the Rap1-binding site lower the frequency of heteroduplex formation at the 5′ end of *HIS4* (WHITE *et al.* 1991). The present study had two purposes: (1) to determine whether the binding of Rap1 stimulated heteroduplex formation at the 3′ end of *HIS4* and in *BIK1* and (2) to determine whether Rap1 binding stimulated individual recombination events unidirectionally (at *HIS4* **or** *BIK1*) or bidirectionally (at *HIS4* **and** *BIK1*).

To address these issues, we constructed two diploid strains with multiple heterozygous insertions of palindromic oligonucleotides. The strain SPY200 (Figure 1) had one chromosome *III* with wild-type alleles of *BIK1* and *HIS4*, and one chromosome *III* with palindromic insertions in *BIK1* (*bik1-IR16*), and in the 5′ and 3′ ends of *HIS4*(*his4-IR9* and *his4-3133*, respectively). The strain SPY202 (Figure 1) was isogenic with SPY200 except that it was homozygous for a mutation in the Rap1-binding site (*his4-51*) upstream of the *HIS4* coding sequence. The mutated Rap1 site contains 4-bp differences from the wild-type Rap1 site that render it unable to bind Rap1 protein (DEVLIN *et al.* 1991). The Rap1-binding site is between *BIK1* and *HIS4* and is approximately equidistant from *bik1-IR16* and *his4-IR9*.

Mutations of the Rap1-binding site reduce the frequency of aberrant segregation of mutations in *HIS4* and in *BIK1*: Table 1 shows the results of measuring meiotic recombination at the three loci in both SPY200 and SPY202. It is clear that SPY202, the strain with the mutant Rap1 site, has about half the amount of aberrant segregation as is found in the strain SPY200, which has a wild-type RAP1 site, for each of the three alleles. As observed in previous studies of mutant alleles caused by palindromic insertions (NAG, WHITE and PETES 1989), the PMS events represented about 65–75% of the total aberrant segregation events. The frequencies of aberrant segregation for the three alleles in SPY200 are similar to those observed in experiments in which the mutations were examined in single-point crosses (NAG and PETES 1991; DETLOFF, WHITE and PETES 1992).

In general, comparable classes of recombinants (for example, 5:3 and 3:5) had similar frequencies. Two exceptions were the 6:2/2:6 ratios for the *bik1-IR16* and *his4-3133* alleles in SPY200. The *bik1-IR16* allele had approximately 4-fold more 6:2 than 2:6 gene conversions ($P < 0.001$ by chi-square analysis) and the *his4-3133* allele had a 3-fold disparity in the same direction ($P < 0.05$). Since these same disparities are not evident for either marker in the 5:3/3:5 class of PMS events (Table 1), the conversion disparity is likely to be a consequence of mismatch repair rather than a difference in the frequency of strand donation for heteroduplex formation. A similar disparity was observed previously for *bik1-IR16*, but not *his4-3133*, in single-point crosses (DETLOFF, WHITE and PETES 1992).

We assume that the aberrant segregation frequency of high PMS alleles is a good approximation of the frequency of heteroduplex formation. An alterative possibility is that the deletion of the Rap1-binding site allows restoration-type repair of mismatches, lowering the frequency of observed aberrant segregants. One argument against this possibility is that crossovers are also reduced by the Rap1-binding site mutation (WHITE *et al.* 1991), an effect that would not be predicted by an alteration in the pattern of mismatch repair.

Patterns of co-PMS and other co-events at the *HIS4* and *BIK1* loci: Although the comparison of aberrant segregation frequencies of SPY200 (wild-type Rap1-binding sites) and SPY202 (mutant Rap1-binding sites) indicates that the Rap1 site stimulates recombination in both of its flanking regions, the results do not demonstrate that single events are stimulated bidirectionally. Conclusions about this issue can be made by studying the patterns of co-events of aberrant segregation. The patterns of co-events, particularly for SPY200, were complex (Figure 2). For SPY200, of 304 tetrads examined, 114 had a single recombination event involving *HIS4* and/or *BIK1*, and 71 tetrads had more than one event involving these markers. We classified tetrads as containing a single recombination event by making the following assumptions: (1) recombination events are initiated by the continuous asymmetric transfer of a single strand from one chromosome to the other; (2) if multiple mismatches are present in a heteroduplex, then these mismatches are repaired in a continuous fashion (*i.e.*, the same strand is used as a template for repair for all mismatches in one heteroduplex); and (3) crossovers (if any) occur adjacent to the beginning or end of a heteroduplex event. Since these assumptions were

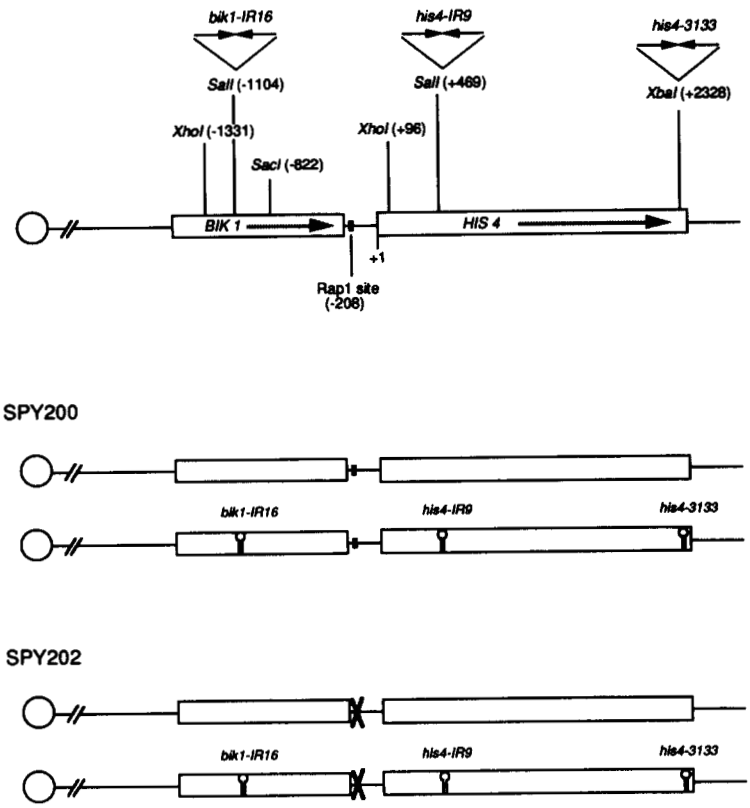


FIGURE 1.—Arrangement of markers in strains SPY200 and SPY202. The top part of the figure shows a map of the *BIK1-HIS4* region of yeast chromosome *III*. The restriction sites are numbered with the first nucleotide of the *HIS4* translation start codon as base pair +1. The three palindromic oligonucleotides are shown above the restriction sites into which they were inserted. The position of the Rap1-binding site is also shown. The direction of transcription is indicated by the arrows on the boxes. The bottom part of the figure represents the chromosome *III* homologs present in each strain. SPY200 has one unaltered chromosome *III* and one chromosome *III* with the three palindromic oligonucleotides inserted at the indicated sites. SPY202 is identical to SPY200 except it is homozygous for 4-bp changes within the Rap1- binding site (*his4-51*); this alteration is indicated by ×'s.

TABLE 1

Effect of the Rap 1-binding site on aberrant segregation at *bik1-IR16*, *his4-IR9* and *his4-3133*

Strain	No. total tetrads	Mutant allele	6:2	2:6	5:3	3:5	4:0	Aberrant 4:4	Other PMS	ABS (%)[a]	GC (%)[a]	PMS (%)[a]	PMS/ABS (%)
SPY200 (WT Rap1 site)	304	*bik1-IR16*	27	6	45	32	1	2	6[b]	39	11	28	71
		his4-IR9	16	7	31	44	0	11	3[c]	37	8	29	79
		his4-3133	16	5	27	31	0	5	4[d]	29	7	22	76
SPY202 (mutant Rap1 site)	295	*bik1-IR16*	7	10	24	16	0	1	0	20	6	14	71
		his4-IR9	6	11	14	19	0	0	0	17	6	11	66
		his4-3133	12	5	12	17	0	0	0	16	6	10	63

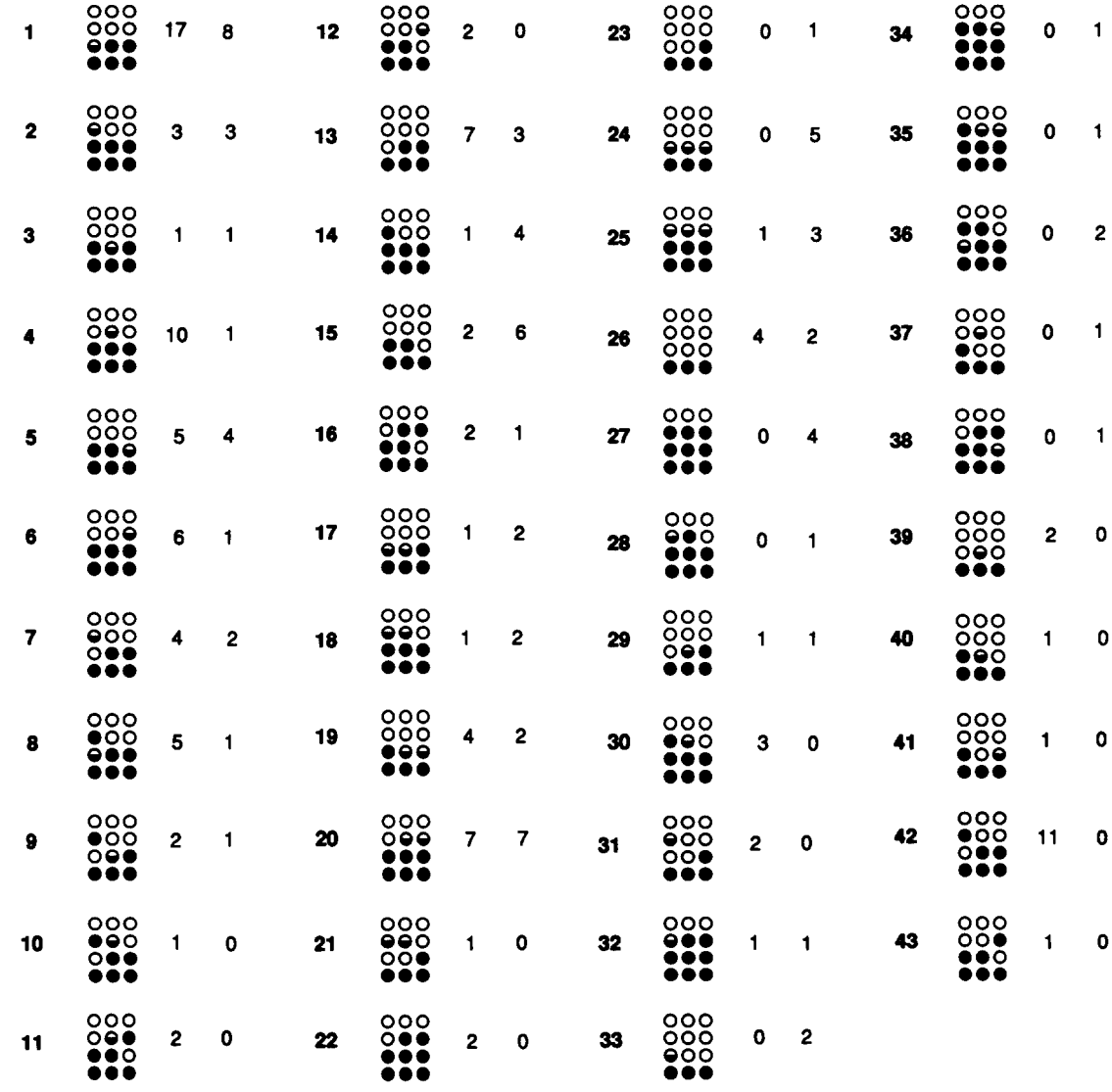
[a] Abbreviations used are: ABS, total aberrant segregations; GC, gene conversions only; PMS, postmeiotic segregations only; WT, wild type.
[b] Two 7:1; two aberrant 6:2; two aberrant 2:6.
[c] One 1:7; one aberrant 6:2; one aberrant 2:6.
[d] One 7:1; two 1:7; one deviant 3:5 (3 PMS events in one tetrad).

based in part on a specific model of recombination, we will later examine the validity of this classification.

In most of the single-event tetrads of SPY200 that have a single sectored colony, the most common classes are those that involve PMS for *bik1-IR16* alone (29 tetrads, classes 1, 2, 7, 8), *his4-IR9* alone (16 tetrads, classes 3, 4, 9–11), *his4-3133* alone (13 tetrads, classes 5, 6, 12), and *his4-IR9* and *his4-3133* together (11 tetrads, class 19, 20). Tetrads involving *bik1-IR16* and *his4-IR9* (3 tetrads, classes 17, 18, 21), or all three markers (1 tetrad, class 25) were much rarer. We conclude that most single recombination events in this region of the chromosome involve either *BIK1* or *HIS4*, but not both loci.

This conclusion is further supported by the data presented in Figure 3, which summarizes the co-PMS data for both the single-event and complex tetrads. In this figure, rather than presenting the number of tetrads with each pattern, we present the number of spore colonies with each pattern, since this method of presentation simplifies subsequent analysis. A spore colony is classified as showing co-PMS if the same spore colony sectors for two (or more) of the heterozygous alleles. There are two patterns of co-sectoring (Figure 4). Since the mutant alleles are on the same chromosome, if a single DNA strand is transferred from one chromosome to the other, one expects to get a sectored colony in which the His^- sector has the mutant alleles for both markers (*cis* orientation). If opposite strands donate, then one expected to get a

A ONE-EVENT TETRADS



Tetrad type	Number found SPY 200	SPY 202	Tetrad type	Number found SPY 200	SPY 202	Tetrad type	Number found SPY 200	SPY 202	Tetrad type	Number found SPY 200	SPY 202
1	17	8	12	2	0	23	0	1	34	0	1
2	3	3	13	7	3	24	0	5	35	0	1
3	1	1	14	1	4	25	1	3	36	0	2
4	10	1	15	2	6	26	4	2	37	0	1
5	5	4	16	2	1	27	0	4	38	0	1
6	6	1	17	1	2	28	0	1	39	2	0
7	4	2	18	1	2	29	1	1	40	1	0
8	5	1	19	4	2	30	3	0	41	1	0
9	2	1	20	7	7	31	2	0	42	11	0
10	1	0	21	1	0	32	1	1	43	1	0
11	2	0	22	2	0	33	0	2			

FIGURE 2.—Patterns of aberrant segregation for mutant alleles *bik1-IR16*, *his4-IR9* and *his4-3133* in strains SPY200 and SPY202. All tetrads with aberrant segregation for *bik1-IR16*, *his4-IR9* or *his4-3133*, or a crossover between these markers is shown. Each type of tetrad is indicated by four rows of three circles. Each row represents a spore colony, and the three circles represent the sectoring pattern of the spore colony for each of the heterozygous loci. Thus, each row represents one of the four chromatids with the gene order being *bik1-IR16* → *his4-IR9* → *his4-3133*. Black circles represent colonies with the mutant phenotype and white circles represent colonies with the wild-type phenotype. Circles that are half black and half white represent sectored colonies. Colonies that co-sectored have either *cis*- or *trans*-sectoring patterns (as indicated in Figure 4). The column to the left of the sectoring patterns represent specific types of tetrads. Numbers to the right represent the number of tetrads of each type observed in SPY200 and SPY202. For example, there were 17 tetrads in SPY200 with the type 1 pattern, 5:3 segregation of *bik1-IR16*, and normal Mendelian segregation of both *his4* alleles. We grouped the tetrads into two groups, one-event tetrads and tetrads with two or more events, based on the criteria discussed in the Results. There were 304 tetrads dissected for SPY200 and 295 tetrads dissected for SPY202.

sectored colony in which the mutant alleles are in opposite sectors (the *trans* orientation). For example, class 17 tetrads represent the *cis* orientation, and class 46 tetrads represent the *trans* orientation.

Several conclusions can be made from Figure 3. First, in SPY200, co-PMS events involving only *his4-IR9* and *his4-3133* (but not *bik1-IR16*) occur more frequently than co-PMS events involving only *bik1-IR16* and *his4-IR9* (35 events *vs.* 9 events; contingency $\chi^2 = 13.4$, $P < 0.01$). It should be pointed out that the physical distance between *bik1-IR16* and *his4-IR9* (1.6 kb) is less than the distance between *his4-IR9* and

B TWO OR MORE EVENT TETRADS

Tetrad type	blk1-IR16 his4-IR9 his4-3133	Number found SPY 200	SPY 202
44		3	0
45		2	0
46		1	0
47		1	0
48		1	0
49		2	0
50		1	0
51		1	0
52		1	0
53		1	0
54		2	0
55		2	0
56		1	0
57		1	0
58		2	0
59		2	1
60		1	0
61		1	0
62		1	0
63		1	0
64		1	0
65		1	0
66		1	0
67		1	0
68		1	0
69		1	0
70		1	0
71		1	0
72		1	0
73		1	0
74		1	0
75		1	0
76		2	0
77		1	0
78		1	0
79		1	0
80		1	0
81		1	0
82		0	1
83		0	2
84		0	1
85		0	1
86		0	1
87		0	1
88		1	0
89		1	0
90		1	0
91		1	0
92		1	0
93		0	1
94		1	0
95		1	0
96		1	0
97		1	0
98		1	0
99		1	0
100		1	0
101		1	0
102		1	0
103		2	0
104		4	0
105		3	0
106		1	0

FIGURE 2.—*Continued*

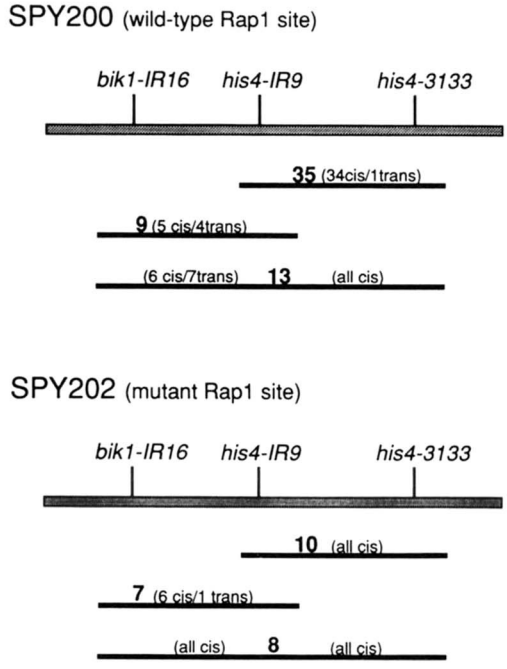


FIGURE 3.—Classes of spore colonies derived from SPY200 and SPY202 that have co-PMS events. This figure shows the number of spore colonies that exhibited co-PMS between any combination of the three alleles. The lines indicate the pattern of co-PMS events for each of the strains. The number in bold above each line gives the total number of spores found with each co-PMS pattern. The *cis* and *trans* designations are explained in Figure 4. For co-PMS events involving all three alleles, the numbers in parenthesis on the left side represent the *cis* and *trans* patterns for *bik1-IR16* and *his4-IR9* , while those on the right side represent the patterns for *his4-IR9* and *his4-3133*.

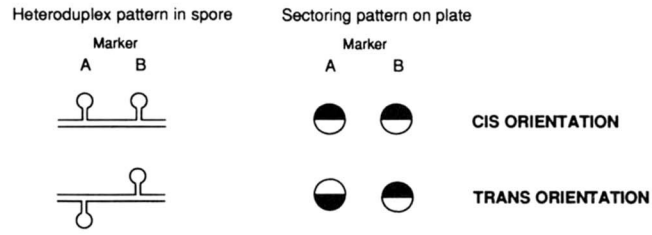


FIGURE 4.—Sectoring patterns for pairs of heterozygous high PMS alleles. In strains SPY200 and SPY202, the mutant alleles involving *HIS4* and *BIK1* are on the same chromosome (Figure 1). If a strand of DNA is transferred continuously from one chromosome to the other, one expects to recover sectored colonies with the *cis* orientation. If the two complementary strands are donated, one forming a mismatch at marker A and the other forming a mismatch at marker B, one expects to recover the *trans* orientation. The mutant spore phenotype is indicated by black and the wild-type phenotype is indicated by white. The two colonies in each row represent the phenotype of a single colony scored separately for marker A and marker B.

his4-3133 (1.9 kb); the Rap1-binding site is located about 700 bp from *his4-IR9* and about 900 bp from *bik1-IR16*. Second, 47 out of 48 of the co-PMS events involving *his4-IR9* and *his4-3133* occur with the *cis* orientation. This result is consistent with these events involving the continuous transfer of a single DNA strand. In contrast, in SPY200, the co-PMS events involving *bik1-IR16* and *his4-IR9* have approximately equal frequencies of the *cis* and *trans* orientations. Third, although the mutation of the Rap1-binding site significantly (contingency $\chi^2 = 12.2$, $P < 0.01$) decreases the frequency of co-PMS events involving the two *his4* mutant alleles, it has substantially less effect on the frequency of co-PMS events involving the *bik1* and *his4* alleles. The effect of the RAP1-binding site mutation on the frequency of co-PMS events involving *bik1* and the *his4* alleles is to significantly reduce (contingency $\chi^2 = 6.86$, $P = 0.01$) the *trans* class of sectoring events without affecting the *cis* class.

The high frequency of co-PMS for *his4-IR9* and *his4-3133* was expected from previous studies involving two-point crosses (DETLOFF and PETES 1992). The reduction of this class by the Rap1-binding site mutation indicates that the heteroduplexes that span these mutant alleles are stimulated by this site. Co-PMS events at sites that individually have high levels of PMS could reflect either a single heteroduplex spanning both sites or independent formation of two heteroduplexes. One can calculate the probability of independent formation of two heteroduplexes by multiplying the probabilities of heteroduplex formation at each individual site.

In SPY200, the frequencies of sectored colonies (among total spore colonies) for individual loci were: 0.076 (93/1216) for *bik1-IR16*, 0.083 (101/1216) for *his4-IR9*, and 0.061 (74/1216) for *his4-3133*. The calculated probability of a spore colony with co-PMS for both *bik1-IR16* and *his4-IR9* is 0.006; the observed frequency of co-PMS for these alleles (including those tetrads in which *his4-3133* also was involved in a PMS event) was 0.018 (Figure 3), approximately threefold higher. This significant (contingency $\chi^2 = 30.2$; $P < 0.001$) difference suggests that at least some of the co-PMS events involving *bik1-IR16* and *his4-IR9* reflect a single initiation event involving both loci. This calculation also indicates, however, that about one-third of the co-PMS events involving both loci may represent independent formation of two heteroduplexes. Similar calculations can be done to estimate the probabilities for co-PMS events involving *his4-IR9* and *his4-3133*, or all three markers in SPY200. The calculated and observed frequencies of co-PMS of *his4-IR9* and *his4-3133* are 0.005 and 0.039, respectively (contingency $\chi^2 = 288$; $P < 0.001$). For co-PMS involving all three markers, the comparable values are 0.0004 and 0.011. Thus, there is approximately an 8-fold excess of co-PMS events involving *his4-IR9* and *his4-3133*, and a 28-fold excess of co-PMS events involving all three markers over the frequencies expected if recombination events were independent at these loci.

A similar analysis can be done for strain SPY202. The frequencies of sectoring for spore colonies for

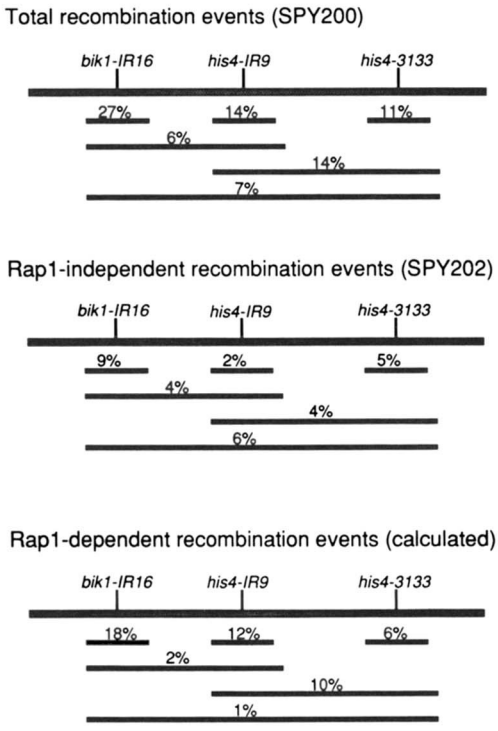


FIGURE 5.—Patterns of aberrant segregation observed for *bik1-IR16*, *his4-IR9*, and *his4–3133* in strains SPY200 and SPY202. This figure shows the percentage of tetrads that involve aberrant segregation (either PMS or conversion) of any of the three markers individually, or co-events involving two or more markers. The top panel shows the recombination events in SPY200 (includes both Rap1-dependent and Rap1-independent events). The middle panel shows the recombination events in SPY202 (Rap1-independent events). The bottom panel shows the inferred pattern of recombination events for RAP1-dependent recombination, calculated by subtracting the SPY202 percentages from SPY200.

each mutant allele were 0.036 (*bik1-IR16*), 0.028 (*his4-IR9*), and 0.025 (*his4-3133*). The calculated (assuming that co-PMS events were independent) and observed frequencies of co-PMS were: 0.001 and 0.013 for *bik1-IR16* and *his4-IR9*; 0.001 and 0.015 for *his4-IR9* and *his4-3133*; 0.00002 and 0.007 for all three markers. Thus, as observed for SPY200, most of the co-PMS events appear to represent single recombination events rather than independent heteroduplex formation at different sites.

Although most of the co-events observed in SPY200 and SPY202 were co-PMS events, we also found tetrads with co-conversion events (two or more markers simultaneously segregating 6:2 or 2:6) or events involving conversion of one marker associated with PMS of another. Type 26 is an example of a tetrad with co-conversion of *bik1-IR16*, *his4-IR9*, and *his4-3133*; type 32 is an example of a tetrad with a PMS event at *bik1-IR16*, associated with a conversion event of *his4-IR9* and *his4-3133*. The co-events exhibited patterns very similar to the co-PMS events (Figure 2).

A summary of all co-segregation events is presented in Figure 5. It was possible to estimate the contribution of the Rap1 site to the recombination patterns in the *BIK1-HIS4* region by subtracting the frequencies found for SPY200 (all recombination events) from those found for SPY202 (Rap1-independent recombination events). It is clear that the majority of Rap1-dependent events include the *BIK1* side of the region (18%) or the *HIS4* side (28%), but usually not both sides (3%) in any one event. In contrast, many of the Rap1-independent events included both BIK1 and HIS4 (10%) compared to events that included only the BIK1 side (9%) or the HIS4 side (11%).

Patterns of crossovers associated with conversion and PMS in strains SPY200 and SPY202: Previous studies in *S. cerevisiae* and other fungi indicate that about half of gene conversion and PMS events are associated with crossing over (FOGEL, MORTIMER and LUSNAK 1981; PETES, MALONE and SYMINGTON 1991). Since there are classes of tetrads in SPY200 that have only one or two tetrads, we will restrict discussion of associated crossovers to the larger classes of single-event tetrads. Of the 29 tetrads that had a PMS event at *bik1-IR16* alone (types 1, 2, 7, 8), 20 were unassociated with a crossover between *bik1-IR16* and *his4-IR9*, and 9 had an associated crossover at this position; the 20 type 1 and 2 tetrads could represent either PMS events unassociated with crossing over or events in which the crossover occurred centromere-proximal to *bik1-IR16*. Of 16 tetrads with a PMS event at *his4-IR9* alone (types 3, 4, 9–11), three had a crossover between *bik1-IR16* and *his4-IR9*, and two had a crossover between *his4-IR9* and *his4-3133*. Of 13 tetrads with a PMS event at *his4-3133* (types 5, 6, 12), two had an associated crossover between *his4-IR9* and *his4-3133*. Of those 11 tetrads with co-PMS for *his4-IR9* and *his4-3133* (Types 19 and 20), none were associated with a crossover between *bik1-IR16* and *his4-IR9*. In summary, about one-quarter of the PMS events in the single-event tetrads of SPY200 were associated with detectable crossovers. This fraction is a minimal estimate of associated crossovers since crossovers occurring centromere-proximal to *bik1-IR16* or centromere-distal to *his4-3133* could not be detected.

Data on crossovers in SPY200 and SPY202 are summarized in Table 2. Since it is difficult to determine the location of crossovers in tetrads with very complex patterns of aberrant segregation, we restricted our analysis to those tetrads in which the segregation patterns could be explained by the formation of one or two heteroduplexes and associated crossovers; thus, the crossover frequencies estimated from Table 2 are minimal estimates. Only those crossovers that occurred at the end of a heteroduplex/conversion tract were considered associated crossovers. The frequency of total tetrads with crossovers in the *bik1-IR16-his4-3133* region in SPY200 (57/304 or 0.19) is significantly (contingency $\chi^2 = 30$; $P <$

TABLE 2

Number of tetrads with *BIK1-HIS4* crossovers in SPY200 and SPY202

Strain	Interval	No. of tetrads	Tetrad types
SPY200	*bik1-IR16-his4-IR9*	43	7–10, 42, 65–67, 69–71, 73, 74, 77, 78, 80, 103–105
	his4-IR9-his4-3133	11	11, 12, 21, 31, 43, 47, 63, 64
	bik1-IR16-his4-3133[a]	3	16, 68
SPY202	*bik1-IR16-his4-IR9*	8	7–9, 37, 38, 83
	his4-IR9-his4-3133	3	36, 86
	bik1-IR16-his4-3133[a]	1	16

Included here are only those tetrads that could be explained by one or two heteroduplex donations plus associated crossover(s). Total number of tetrads scored was 304 for SPY200 and 295 for SPY202.

[a] Includes only those tetrads with a crossover between *bik1-IR16* and *his4-3133* that could not be mapped to a smaller discrete interval.

0.001) higher than that observed for SPY202 (12/295 or 0.04). Thus, the mutation of the Rap1-binding site substantially reduces the level of crossovers in this region of the chromosome. This result supports the previous conclusion that the Rap1-binding site mutation reduces crossovers in the *LEU2-HIS4* interval (WHITE *et al.* 1991).

Rap1-dependent heteroduplexes involve transfer of the nontranscribed strand: Previously, we showed that PMS events at *HIS4* were preferentially the result of heteroduplexes formed by donating a nontranscribed strand (NAG and PETES 1990). In order to find out which strand is donated, one must determine whether a wild-type or mutant gene is a donor during heteroduplex formation, and whether a sectored colony is derived from a spore that is phenotypically His^+ or His^-. For example, if a tetrad had a $5^+:3^-$ segregation pattern and the sectored colony is derived from a His^+ spore, one could infer that a wild-type gene has donated a transcribed strand to a mutant gene. The experimental protocol for determining the phenotypes of spores is described in NAG and PETES 1990.

We examined the specificity of strand transfer in DNY11 [heterozygous for *his4-lop* (a high PMS allele), wild-type UAS; NAG, WHITE and PETES (1989)] and MW118 [heterozygous for *his4-lop*, homozygous for *his4-51* (mutant Rap1 site), WHITE *et al.* (1991)]. Both strains were isogenic with SPY200 and SPY202, except for alterations introduced by transformation. Of 232 tetrads derived from DNY11, 93 sectored colonies were derived from donation of the nontranscribed strand, and 15 were derived from donation of the transcribed strand. This 6:1 preference in favor of the nontranscribed strand was similar to that observed previously with a different palindromic insertion in *HIS4* (NAG and PETES 1990). In 320 tetrads derived from MW118, 24 sectored colonies were derived from the nontranscribed strand and 12 were derived from the transcribed strand. The bias in favor of the nontranscribed strand issignificantly ($\chi^2 = 7$, $P < 0.01$) reduced by the mutation of the Rap1-binding site. The simplest explanation of this result is that almost all of the heteroduplexes that are stimulated by the binding of Rap1 involve transfer of the nontranscribed strand.

DISCUSSION

The complex segregation patterns observed for heterozygous markers in *BIK1* and *HIS4* in SPY200 and SPY202 indicate that meiotic recombination in this region of the yeast chromosome involves multiple initiation sites for formation of heteroduplexes. There are two straightforward arguments for at least two types of heteroduplex in this region. First, we have shown previously that about 80% of heteroduplexes that include the 5′ end of *HIS4* involve transfer of the nontranscribed strand, and 20% involve transfer of the transcribed strand (NAG and PETES 1990; DETLOFF, SIEBER and PETES 1991). Second, certain classes of co-events (such as events involving all three markers) are found at significantly different frequencies for Rap1-dependent and Rap1-independent heteroduplexes. Below, we will first discuss Rap1-independent heteroduplexes, followed by a discussion of the Rap1-dependent heteroduplexes. We will also examine the mechanism by which the Rap1-binding site stimulates recombination.

Rap1-independent heteroduplexes: In this section, we will discuss both the initiation of Rap1-independent heteroduplexes and patterns of mismatch repair in such heteroduplexes. Our discussion will also include data derived from isogenic strains used in our previous studies of recombination in the *BIK1-HIS4* region (NAG, WHITE and PETES 1989; WHITE *et al.* 1991; DETLOFF and PETES 1992; DETLOFF, WHITE and PETES 1992). The frequencies of formation of Rap1-independent heteroduplexes are analyzed by measuring the frequency of aberrant segregation of high PMS alleles in strains in which the Rap1-binding site has been deleted (*his4-Δ52*) or mutated (*his4-51*).

TABLE 3

Calculated amount of restoration-type repair for mismatches located in Rap1-dependent and Rap1-independent heteroduplexes

Heteroduplex type	Mutant locus	ABS tetrads (%) (high PMS alleles)[a]	ABS tetrads (%) (low PMS alleles)[b]	Restoration tetrads (%)[c]	Restoration-type repair (%)[d]
Rap1-dependent and Rap1-independent heteroduplexes (wild-type strains)[e]	*bik1*	41	26	15	36
	5′ his4	35	31	4	11
	3′ his4	33	20	13	37
Rap1-independent heteroduplexes (strains with mutant Rap1-binding site)[f]	*bik1*	20	11	9	45
	5′ his4	19	13	6	31
	3′ his4	16	16	0	0
Rap1-dependent heteroduplexs (calculated)[g]	*bik1*	21	15	6	29
	5′ his4	16	18	0	0
	3′ his4	17	4	13	76

[a] Average for high PMS alleles (NAG, WHITE and PETES 1989; WHITE *et al.* 1991; DETLOFF and PETES 1992; DETLOFF, WHITE and PETES 1992). The calculations were based on the aberrant segregation frequency of a single allele for each locus, except for the 5′ *HIS4* locus (average of 3 alleles).

[b] Average for low PMS alleles (NAG, WHITE and PETES 1989; WHITE *et al.* 1991; DETLOFF, WHITE and PETES 1992). The calculations were based on the aberrant segregation frequency of a single allele for each locus.

[c] Difference in aberrant segregation frequency (percent ABS high PMS alleles minus percent ABS low PMS alleles).

[d] Percent restoration tetrads divided by percent ABS for high PMS alleles.

[e] Summary of data for all strains with wild-type Rap1-binding site.

[f] Summary of data for all strains with mutant Rap1-binding site (*his4-Δ52* or *his4-51*).

[g] Entries were calculated for percent ABS (high and low PMS alleles) by subtracting the percent ABS observed for strains with the RAP1 binding site mutation from the percent ABS observed with the wild-type strains.

We find a similar level of heteroduplex formation (aberrant segregation) at *BIK1* (20%) and at the 5′ and 3′ ends of *HIS4* (19% and 16%, respectively; Table 3). Thus, the level of Rap1-independent heteroduplexes is approximately the same across the *BIK1-HIS4* region.

The frequencies of aberrant segregation for low PMS markers in the absence of the Rap1 site are also summarized in Table 3. As discussed in the Introduction, a difference in the aberrant segregation frequencies for a high PMS marker and a low PMS marker at the same position in a gene is an indication of the amount of restoration-type repair for the well repaired mismatch (DETLOFF, WHITE and PETES 1992). Thus, since the high PMS markers have a significantly higher aberrant segregation frequency for mutations at the 5′ end of *HIS4* and in *BIK1*, we conclude that mismatches at these positions in Rap1-independent heteroduplexes have a significant level of restoration-type repair. The calculated frequency of the **total** tetrads that represent restoration events for low PMS alleles in strains with mutations of the Rap1-binding site are shown in Table 3. In contrast, since the high PMS marker at the 3′ end of *HIS4* has the same frequency of aberrant segregation as that found for the low PMS marker, we conclude that mismatches at the 3′ end of *HIS4* in Rap1-independent heteroduplexes do not have a significant level of restoration-type repair. It is possible that there is a Rap1-independent site located near the 3′-end of *HIS4* that both initiates heteroduplex formation and influences the repair gradient in a manner analogous to the Rap1-dependent site. These conclusions about the level of restoration are based on two assumptions: (1) that the frequency of heteroduplex formation is the same for both high PMS and low PMS alleles at the same position in a gene, and (2) mismatches that are inefficiently corrected by conversion-type repair are also inefficiently corrected by restoration-type repair.

The last issue that we will discuss concerning RAP1-independent heteroduplexes is the site(s) of initiation. Since the level of heteroduplex formation in single-point crosses was approximately the same throughout the *HIS4-BIK1* region, one simple interpretation of these data is that heteroduplexes initiate at a single-site downstream of the 3′ end of *HIS4* or upstream of *BIK1* and propagate through the entire *HIS4-BIK1* region. If this model is correct, one should find frequent co-events involving all three markers in multipoint crosses. Although such classes of aberrant segregants were seen in SPY202 (for example, class 25), these classes did not represent a majority of aberrant tetrads. Thus, the pattern of co-events is consistent with two or more Rap1-independent initiation sites and we cannot exclude the possibility that the Rap1-independent events are initiated randomly.

Rap1-dependent heteroduplexes: Although we can examine directly the characteristics of the Rap1-independent heteroduplexes by analyzing strains with mutations of the Rap1-binding site, we have no method of directly examining the Rap1-dependent heteroduplexes. Consequently, our information con-

cerning Rap1-dependent heteroduplexes is obtained by subtracting the contribution of the Rap1-independent heteroduplexes from those heteroduplexes observed in wild-type strains as shown in Table 3 and Figure 5. These calculations indicate that the frequency of Rap1-dependent heteroduplex formation is similar at *BIK1* and the 5′ and 3′ ends of *HIS4*. Calculations of the amount of restoration-type repair indicate that the same mismatches in Rap1-dependent and Rap1-independent heteroduplexes can be repaired with a different bias for restoration-type and conversion-type repair (Table 3). For example, at the 3′ end of *HIS4*, most of the mismatches (76%) in Rap1-dependent heteroduplexes are corrected by restoration-type repair, whereas none of the mismatches in Rap1-independent heteroduplexes are corrected by restoration-type repair. Since our conclusions about restoration-type repair at the 3′ end of *HIS4* are based on comparisons of two alleles, we cannot exclude the possibility that other mismatches at this position would show different ratios of conversion-type to restoration-type repair. At the 5′ end of the gene, however, multiple alleles were examined and this type of variation was not seen (DETLOFF, WHITE and PETES 1992).

The simplest explanation of the stimulation of recombination by Rap1 is that the binding of the protein stimulates heteroduplex formation at the Rap1-binding site. Alternatively, the Rap1 protein could act as an enhancer of recombination that stimulates heteroduplex formation at a site(s) that is not contiguous with the Rap1-binding site. These data (summarized in Figure 5) indicate that binding of the RAP1 protein significantly stimulates heteroduplexes that involve single-site events at *bik1-IR16*, *his4-IR9* and *his4-3133*, as well as stimulating heteroduplexes that include both *HIS4* markers in one event. The binding of Rap1, however, does not stimulate formation of co-events involving *BIK1* and one or both *HIS4* markers (with the exception of *trans* co-PMS events), or those co-events involving all three markers; such events presumably result from Rap1-independent heteroduplex formation.

The stimulation of single-site heteroduplex formation at *BIK1* and the 5′ end of *HIS4*, as well as stimulation of double-site events at the 5′ and 3′ ends of *HIS4*, is consistent with the possibility that Rap1-dependent heteroduplexes initiate at the Rap1-binding site and can be propagated either toward *BIK1* or toward *HIS4* from that site. The Rap1-induced stimulation of heteroduplexes that include only the marker at the 3′ end of *HIS4*, however, is not easily explained by an initiation event that occurs at the Rap1 site. It is possible that, although most of the Rap1-dependent heteroduplexes are initiated near the Rap1-binding site, the bound Rap1 protein can also stimulate the initiation of heteroduplexes at a distance.

Segregation patterns in SPY200 consistent with formation of a double-strand break at the Rap1-binding site: At two loci in *S. cerevisiae*, hotspots for meiotic recombination have been associated with formation of a meiosis-specific double-stranded break in the DNA (SUN *et al.* 1989; CAO, ALANI and KLECKNER 1990). At the *ARG4* locus, the ends of the break appear to be processed into single-stranded tails by the action of a 5′ to 3′ exonuclease (SUN, TRECO and SZOSTAK 1991). Based on these data, as well as data from other labs indicating that most conversion events reflect repair of mismatches in heteroduplexes, a modified version of the original double-strand break repair model of recombination (SZOSTAK *et al.* 1983) was generated (Figure 6, after SUN, TRECO and SZOSTAK 1991). If the Rap1-binding site represents the site of a double-strand break and if the broken chromosome is processed symmetrically, then the expected most common classes of recombinant tetrad would be those with an apparent crossover between *bik1-IR16* and *his4-IR9*, with sectored spore colonies in recombinant configurations.

We found the pattern expected by the model shown in Figure 6 for nine recombinant tetrads (types 103–105) out of a total of 185 recombinant tetrads derived from SPY200. Since the Rap1 site stimulates recombination at *BIK1* and *HIS4* about twofold, this fraction is much smaller than that expected if most of the Rap1-stimulated events were a consequence of a double-strand break at the Rap1-binding site that was then processed in a symmetric manner. The simplest interpretation of our data is that the Rap1-binding site does not stimulate heteroduplex initiation by the mechanism proposed for the *ARG4* hotspot. A double-strand break repair model in which the invading ends are derived from a single DNA strand (Figure 10 of SZOSTAK *et al.* 1983) is also not supported by our data, as this model predicts a large number of *bik1-IR16-his4-IR9* co-PMS events with the *cis* orientation; such events were not Rap1-dependent (Figure 3). In experiments similar to our analysis of *HIS4*, L. GILBERTSON and F. STAHL (personal communication) have found frequent co-PMS events with the *trans* orientation for markers flanking the *ARG4* hotspot. In addition, SCHULTES and SZOSTAK (1990) found frequent co-conversion for markers flanking the *ARG4* hotspot.

The nine tetrads that have the pattern predicted by the double-strand break repair model could represent recombination events initiated by a double-strand break at the Rap1-binding site. Alternatively, the same pattern could be generated by two heteroduplexes forming independently, one involving *bik1-IR16* alone, and one involving *his4-IR9* and/or *his4-3133*. If the two PMS events involve donating strands from

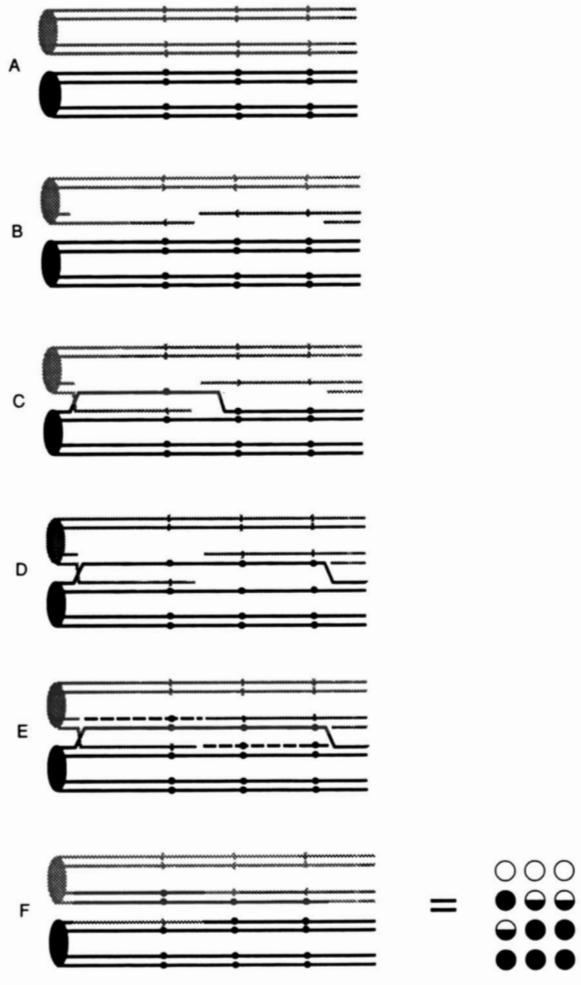


FIGURE 6.—The modified double-strand break repair model of recombination (SUN, TRECO and SZOSTAK 1991) and the aberrant segregation patterns predicted from this model. In A, the two sets of homologs are about to undergo meiotic recombination. The chromosome with the mutant alleles is represented by black lines and the chromosome with wild-type alleles is represent by zig-zag lines. The three heterozygous markers are indicated by small circles or small bars. In B, a double-strand break has occurred, followed by symmetric excision of the 5′ ends of the broken chromosome, generating 3′-overhanging single strands. In C, one of these strands invades the homologous chromosome forming a heteroduplex, displacing the resident strand. In D, the displaced strand pairs with the other broken end, forming a second heteroduplex. In E, repair synthesis fills in the remaining gap, and, in F, the cross-strands are cleaved in a mode that retains the parental combination of flanking markers. This pattern would lead to a tetrad of the type 105 (Figure 2).

different chromatids, then we assume that independent formation of two heteroduplexes is involved. Five such tetrads (types 59, 73, 78, 79, 80) were observed. In addition, the type 66 tetrad is most easily explained as two independent heteroduplexes, since three chromatids have recombinant configurations of markers. Consequently, at least some of the tetrads that have the double-strand break configuration are likely to represent independent formation of two heteroduplexes.

Alternative mechanisms for Rap1-stimulated heteroduplex induction: As described above, we have shown the binding of the Rap1 protein to a site upstream of *HIS4* stimulates heteroduplex formation involving flanking markers on both sides of the binding site. At the 5′ end of *HIS4*, the Rap1-dependent heteroduplexes involve almost exclusively transfer of the nontranscribed strand. In addition, from experiments in which the Rap1-binding site is heterozygous, it is clear that the chromosome with the Rap1-binding site acts as a recipient in the strand transfer event (WHITE *et al.* 1991). Finally, we have found that the Rap1-binding site itself is not sufficient for hotspot activity. When the Rap1-binding site was moved downstream of *HIS4*, no enhanced level of recombination was observed for a marker at the 3′ end of *HIS4* (WHITE *et al.* 1991).

Most of our data on Rap1-stimulated recombination are consistent with a version of the RADDING (1982) model of recombination shown in the top part of Figure 7. In this model recombination is initiated by a single-strand nick. The binding of Rap1 to its binding site between *BIK1* and *HIS4* stimulates the entry of a 5′ to 3′ exonuclease. In most tetrads, this nuclease degrades the nontranscribed strand of *HIS4* or the transcribed strand (a prediction not yet tested) of *BIK1*, producing a gap that is invaded by a strand from the homologous chromosome. In a small fraction of the tetrads, nucleases degrade both strands, resulting in a double-strand break or a DNA molecule with two closely spaced gaps. An alternative model consistent with the data is an asymmetric version of the double-strand break model in which a nuclease degrades primarily in one direction or the other, producing only one long single-stranded tail in any one event (Figure 7, bottom). Less frequently, symmetric processing might occur to give the recombination pattern found in tetrad types 59, 73, 78, 79 and 80.

The difference in the ratio of conversion- to restoration-type repair for mismatches in Rap1-dependent and Rap1-independent heteroduplexes may reflect a difference in the position or type of DNA lesion that initiates the two types of heteroduplexes. Mismatches at the 5′ end of *HIS4* in Rap1-dependent heteroduplexes have little restoration repair (Table 3). In the model shown in the top part of Figure 7, if a gap in the nontranscribed strand initiates Rap1-dependent *HIS4* recombination and also acts as a loading site for a mismatch repair system that preferentially removes mismatches from the unnicked strand, one would see only conversion-type repair near that loading site. In prokaryotes, however, it is the nicked strand that is excised from a heteroduplex containing a mismatch (MODRICH 1991). One intriguing feature of the asymmetrically processed double-strand break model shown in Figure 7 is that a nick-directed mismatch

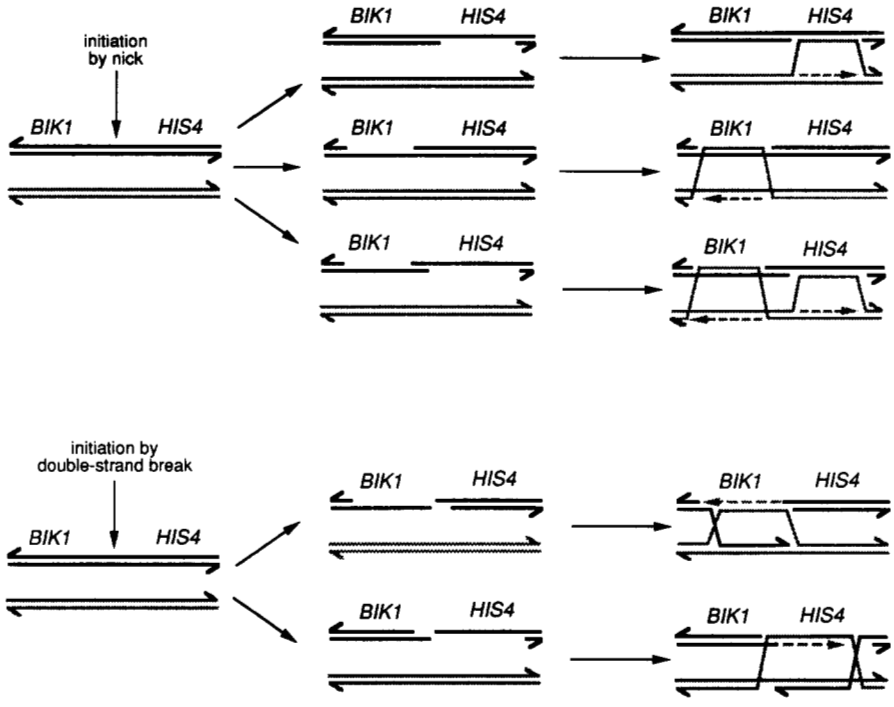


FIGURE 7.—Proposed mechanisms of heteroduplex initiation at the Rap1-binding site. We propose that binding of the Rap1-binding protein stimulates the production of a single-strand nick (top part of figure) or double-strand break (bottom part of figure) that is asymmetrically processed by a 5′ to 3′ nuclease. If the lesion is a nick, the nuclease forms a gap either by digesting the nontranscribed strand of *HIS4* (A) or by digesting the transcribed strand of *BIK1* (B). Much less frequently, both strands are digested resulting in a chromosome with two gaps that can be invaded by two different strands derived from the opposite homolog (C). If the lesion is a double-stand break, the nuclease degrades either the transcribed strand of *BIK1* (D) or the nontranscribed strand of *HIS4* (E). The resulting intermediates can be further processed as discussed by RADDING (1982) and SZOSTAK *et al.* (1983).

repair event would preferentially result in gene conversion rather than restoration.

In summary, the patterns of aberrant segregation observed in our study indicate that the hotspot of recombination caused by Rap1 binding does not represent a site at which a symmetrically processed double-strand break occurs. The patterns are consistent with models involving single-strand gap formation or asymmetrically processed double strand breaks (Figure 7). It is possible that there are several types of lesions that are used to initiate meiotic recombination in yeast, and the relative importance of these lesions is different at different loci.

The research was supported by National Institute of Health (NIH) grant GM24110 and NIH National Research Service Award GM13805-01 (S.E.P.). We thank D. NAG, C. NEWLON, K. ARNDT, P. DETLOFF and J. TRUEHEART for strains and plasmids used in this study. We thank other members of the PETES laboratory, M. LICHTEN, L. GILBERTSON (and other members of the STAHL laboratory), G. SMITH and P. DETLOFF for critical reading of this manuscript. We thank L. GILBERTSON for communicating unpublished data and P. PUKKILA for helpful discussions regarding recombination models.

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Communicating editor: G. R. SMITH