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A DNA Sequence Conferring High Postmeiotic Segregation Frequency to Heterozygous Deletions in *Saccharomyces cerevisiae* Is Related to Sequences Associated with Eucaryotic Recombination Hotspots

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The meiotic behavior of two graded series of deletion mutations in the *ADE8* gene in *Saccharomyces cerevisiae* was analyzed to investigate the molecular basis of meiotic recombination. Postmeiotic segregation (PMS) was observed for a subset of the deletion heterozygosities, including deletions of 38 to 93 base pairs. There was no clear relationship between deletion length and PMS frequency. A common sequence characterized the novel joint region in the alleles which displayed PMS. This sequence is related to repeated sequences recently identified in association with recombination hotspots in the human and mouse genomes. We propose that these particular deletion heterozygosities escape heteroduplex DNA repair because of fortuitous homology to a binding site for a protein.

Meiotic recombination is the fundamental process which is responsible for the reassortment of genetic information between paired homologous chromosomes in zygotic organisms. The physical recombination event takes place after premeiotic DNA replication but before the first nuclear division (1). At that time, each locus is represented on eight DNA strands, i.e., two double-stranded copies of each homolog. Ordinarily, interaction between homologs results in a detectable exchange of information. This exchange can involve one or two strands of DNA and can be reciprocal or nonreciprocal. A nonreciprocal exchange is termed a gene conversion. Because reciprocal and nonreciprocal exchanges are nonrandomly associated in meiosis, gene conversion can be regarded as a signature of the recombination process (5). We study recombination in *Saccharomyces cerevisiae* because of the powerful confluence of available molecular and genetic technologies. In particular, tetrad dissection of sporulated, appropriately marked diploid strains, followed by replica plating of the resulting four undisturbed ascosporal colonies, allows us to visualize the ultimate fates of each of the eight DNA strands which were present before the meiotic divisions. In addition, replacement of resident alleles by in vitro-modified sequences is now a routine procedure (2).

Postmeiotic segregation (PMS) is detected phenotypically as the sectoring of a single heterozygous marker in a haploid ascosporal colony and represents marker segregation at the first postmeiotic mitosis. Presumably, such segregation results when the spore contains uncorrected heteroduplex DNA which encompasses the heterozygosity. PMS at *ADE8* can be visualized as red and white half sectors caused by the interaction of *ADE2* and *ADE8*. PMS in yeast was first observed by Esposito in an *ade8-18/ADE8* heterozygote (3).

In a previous analysis, it was found that PMS frequency can be correlated with the potential heteroduplex DNA mismatch (16) generated by the interacting alleles. Furthermore, among those analyzed, the mutant allele displaying the highest PMS frequency (*ade8-18*) was determined to harbor a 38-base-pair (bp) deletion. Since previous studies showed that large deletions and frameshift mutations do not display appreciable PMS levels (6), this result raised the question whether the high PMS value observed for *ade8-18* would also be found for small deletions in general or whether a specific sequence was responsible for high PMS levels. Accordingly, series of *Bal*31 deletions were generated at two sites in the *ADE8* gene. An analysis of these deletion heterozygosities in an isogenic background failed to demonstrate any dependence of PMS frequency on deletion length. Instead, those deletions displaying appreciable PMS shared a common sequence spanning or directly adjacent to the deletion breakpoint. This shared sequence is related to sequences shown to be associated with recombination hotspots in other organisms, *chi* in *Escherichia coli* (13) and related minisatellite sequences identified first in an intron of human myoglobin (8) and later in the I region of the mouse major histocompatibility complex (10, 15).

MATERIALS AND METHODS

Deletion constructions. A plasmid bearing a 4-kilobase *ADE8* insert at the *Bam*HI site in the yeast-bacterial shuttle vector YRp17 (4) was opened at a unique site within the *ADE8* coding region, *Xho*I for one deletion series, and *Hpa*I for the other (relevant restriction sites are indicated in Fig. 2). Each DNA sample was digested with *Bal*31 nuclease under conditions calculated to degrade approximately 5 bp/min from each end of the fragments. Aliquots were removed into EGTA to terminate the reaction after 1, 3, 5, 15, and 30 min. Restriction mapping of the digestion products with *Sal*I confirmed the approximate degradation rate. Each reaction aliquot was extracted three times with phenol and once with chloroform-isoamyl alcohol (24:1) and then ethanol precipitated. T4 DNA polymerase was used to repair

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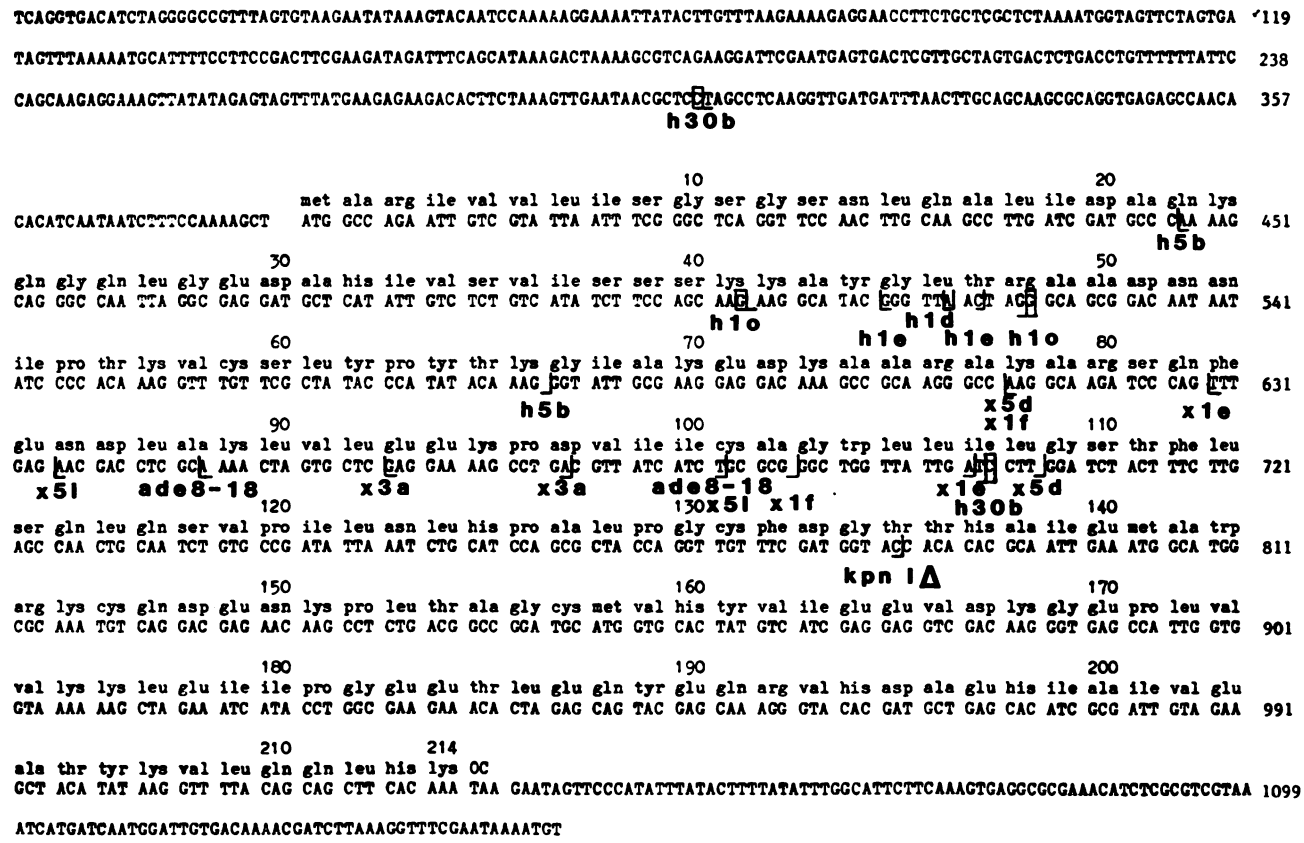

FIG. 1. Nucleotide sequences of *ADE8* deletion mutations. The nucleotide sequence of the *ADE8* gene is shown. Endpoints of deletions are indicated by brackets. The 5′ endpoint for Δ*kpnI* is off the scale of the figure. The sequence for *x30a* was not determined. Deletions *h30b* and *h1o* are indeterminate by 1 bp at each endpoint because of the local sequences.

staggered DNA ends. The DNA was then religated with T4 DNA ligase under conditions favoring unimolecular reactions. Individual deletions were cloned as ampicillin-resistant colonies in the bacterial strain HB101.

After initial characterization of the deletions by restriction mapping, plasmid DNA isolated in minipreparations by the boiling method (7) was introduced (9) into a yeast host strain with an *ade2-1 ADE8 ura3-52* genotype (see below). Transformants were selected on medium lacking uracil and then grown nonselectively on YEPD. Subsequent replica plating to a medium lacking uracil revealed stable transformants. These stable transformants were streaked onto YEPD again, and monitored for the appearance of rare white colonies. Such white colonies were screened for the simultaneous loss of both uracil prototrophy and *ADE8* function in order to confirm the loss of the plasmid and replacement of the resident wild-type *ADE8* allele with the in vitro-generated deletion. The location and approximate size of the deletion after transfer to the yeast chromosome was confirmed by Southern analysis (14). The nucleotide sequence of each deletion allele was determined by subcloning the appropriate fragment of the shuttle plasmid into bacteriophage M13mp18 or M13mp19 and submitting the resulting clones to dideoxy sequencing (12).

Strain construction and data collection. The recipient yeast strain for transformation with deletion-bearing plasmids was JHW134-3-5Aa, with the genotype *ade2 his4-519 leu2-3,112 SUF3 ura3-52 lys1-1 met13*. The resulting deletion strains were then crossed to JHW154-3-21Dα with the genotype *ade2 his4-519 leu2-3,112* Δ*trp1 arg4-16 thr1 tyr1-1 rna3*. Individual zygotes were isolated by micromanipulation and allowed to grow into a 3-mm colony on glucose nutrient agar. The diploid strain was then patched onto glucose nutrient agar for overnight growth and replica printed to potassium acetate sporulation medium while it was growing vigorously. Sporulation was generally complete within 48 h. Ascal walls were removed with glusulase, and after being washed the resulting suspension was used for tetrad dissection for up to 1 week. Data were collected for several zygotes per cross. These are presented in combined form, since there were no significant differences among such data subsets.

The diploid strains analyzed are heterozygous at 11 loci on seven different chromosomes. This large number of heterozygosities allowed us to detect and eliminate false tetrads with a high level of confidence. The *SUF3* and *rna3* markers were chosen because they flank *ADE8*. *SUF3* segregations were scored on plates lacking histidine by monitoring suppression of the homozygous *his4-519* frameshift mutation. Segregation of *rna3* was followed at 37°C. Due to the presence of this temperature-sensitive mutation, dissected asci were incubated at 25°C for 4 to 5 days to allow spore colony formation. The positive and negative control alleles for PMS frequency were *arg4-16* and *met13*, which display PMS frequencies of 33 and 0.1%, respectively, among aberrant tetrads (6).

Nomenclature. New *ADE8* deletion alleles carry a three-part designation. First, the letter *h* or *x* indicates whether the deletion is centered at the *Hpa*I or *Xho*I site. The number

TABLE 1. Segregation data for *ADE8* heterozygosities[a]

Allele	Length of deletion (bp)	No. of tetrads	No. of gene conversion events				Aberrant frequency (%)	PMS frequency (%)
			6:2	2:6	5:3	3:5		
x3a	14	445	13	10			5.2	
ade8-18	38	373	7	3	5	12	7.2	63.0
x5l	49	342	5	4	1	1	3.2	18.2
x1e	73	421	13	7			4.8	
x1f	75	592	17	4	5	1	4.6	22.2
x5d	93	366	7	7	2		4.4	12.5
x30a		537	7	16			4.3	
h1d	1	425	33	22			12.9	
h1e	8	361	21	18			10.8	
h1o	21	535	4	14			3.4	
h5b	134	186	2	9			5.9	
h30b	398	197	3	10			6.6	
Δ*kpn1*		504	5				1.0	
x total		3,076	69	51	13	14	4.8	18.4
h total		1,704	63	73			8.0	
All total		5,284	137	124	13	14	5.5	9.4

[a] Gene conversions are separated into 6+:2− and 2+:6− events, whereas PMS events are separated into 5+:3− and 3+:5− segregation patterns. The basic conversion frequency, or aberrant tetrad frequency, is the proportion of total segregations departing from the normal 4+:4− pattern. PMS frequency is the proportion of aberrant tetrads which display PMS.

following the letter represents the duration of the *Bal*31 nuclease treatment in minutes. The final letter reflects sequential labeling of clonal plasmid isolates from bacteria. For example, *x3a* is an allele with a deletion at the *Xho*I site, which was generated by a 3-min *Bal*31 digest, and was the first plasmid analyzed among those isolated in the *x3* series.

RESULTS

Nucleotide sequences of deletion alleles. The nucleotide sequence of the *ADE8* gene (16) is displayed in Fig. 1. The breakpoints for each deletion analyzed are indicated. Deletion *x30a* was not sequenced, and the 5′ breakpoint for Δ*kpn1* is off the scale for this figure. The extent of the *ade8-18* deletion is shown for comparison. Several breakpoints are shared. The *x5l* and *ade8-18* deletions share the same 3′ endpoint, whereas the *x1f* and *x5d* deletions begin at the same base.

Meiotic behavior of heterozygous deletions. The results of meiotic segregation of the various *ADE8* deletion heterozygosities in a total population of 5,284 unselected tetrads with four surviving spores are presented in Table 1. Data are presented for 13 different alleles, including 11 *Bal*31 deletions, a single 1.2-kilobase deletion between *Kpn*I sites, and *ade8-18* which was transferred into the isogenic series by gene replacement.

In accord with our previous results (6), the frameshift (*h1d*) and large deletions (*x30a*, *h30b*, and Δ*kpn1*) did not

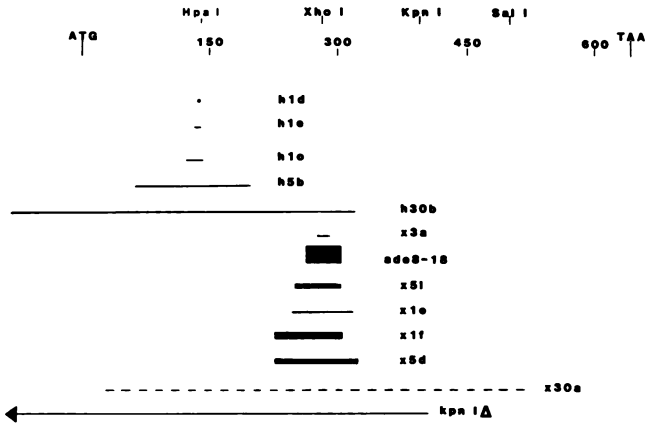

FIG. 2. Independence of PMS frequency from deletion size. The location of each deletion mutation analyzed is indicated. The thickness of each line reflects relative PMS frequencies.

|G C G C G G G C T G G T T	x5l	18%
|G C G C G G G C T G G T T	ade8-18	63%
G C A A G G G C C |G G C T	x1f	22%
G C A A G G G C C |G G A T	x5d	13%
G C G G G C G G T	Invariant	
G C PuX G G G C PyG .G X T	Consensus	

FIG. 3. Alignment of junction sequences for deletions displaying PMS. Nucleotide sequences 14 bp on either side of the deletion breakpoint were aligned for those mutations displaying PMS. The best alignments, along with PMS frequencies, are shown. Because of shared junction points, all of *x5l* or *ade8-18* and the left half of *x1f* or *x5d* should be disregarded when deriving the consensus sequence.

TABLE 2. Score for best alignment of each sequenced deletion allele with the consensus sequence[a]

Allele	Alignment score
x3a	5.5
ade8-18	10
x5l	10
x1e	5
x1f	10
x5d	10
h1d	6
h1e	6
h1o	5.5
h5b	6
h30b	4.5

[a] Homology search among the breakpoint sequences. The best alignment in the 28 bp which symmetrically span the deletion breakpoints with the consensus sequence derived in Fig. 3 is given a score reflecting homology. The 28-bp sequence was surveyed in groups of 13 bp. Each match to a specified base was awarded 1 point, and each purine-purine or pyrimidine-pyrimidine match at positions which were designated in the consensus sequence as purine or pyrimidine was awarded 0.5 point. Because no points could be awarded at the two nonspecified (x) positions, and two positions can each receive only 0.5 point, a perfect match to the consensus is 9 + 0 + 0 + 0.5 + 0.5 = 10.

display PMS, although a total of 96 gene conversions were observed among 1,663 tetrads involving these four heterozygosities.

No PMS events were observed for any of the deletions generated at the *Hpa*I site, where 1,704 combined tetrads exhibited 136 gene conversions. In contrast, three of six *Bal*31 deletions at the *Xho*I site displayed detectable PMS frequencies.

A scaled representation of the *ADE8* coding region and the deletions analyzed in this study is shown in Fig. 2. The thickness of the line depicting the deletion extent is proportional to the PMS frequency. Since *ade8-18* and *x5l* share 3′ breakpoints and the *x1e* deletion removes 18 bases 3′ to the common breakpoint, we might infer that these 18 bases are involved in the high PMS values for *ade8-18* and *x5l* when compared to no detected PMS events for *x1e*. However, the *x5d* deletion removes an additional 5 bases which are 3′ to the *x1e* breakpoint while displaying 12.5% PMS among aberrant tetrads.

A consensus sequence for PMS of deletions. Since the results given above rule out simple interpretations where PMS frequency for deletion heterozygosities depends on deletion size or deletion location within the *ADE8* gene, the nucleotide sequences which span the deletion breakpoints were compared. It was found that when a region of 28 bp which symmetrically spans each deletion is analyzed, the four deletion alleles which display PMS share significant homology. A 13-base consensus sequence can be identified, with 9 bases which are invariant in location and identity,

AG G CA GGC A GG C A	Mouse	I-J Repeat
GC PuX G GGC PyG G X T	Yeast	Deletion PMS
AG G TG GGC A GG A X	Human	Minisatellite
GC T GG T G G	Bacteria	Chi

FIG. 4. Comparison of sequences which are involved in recombination. The consensus sequence derived in Fig. 3 is compared to recombination hotspot sequences from mouse (10, 15), human (7), and bacterial (13) DNA.

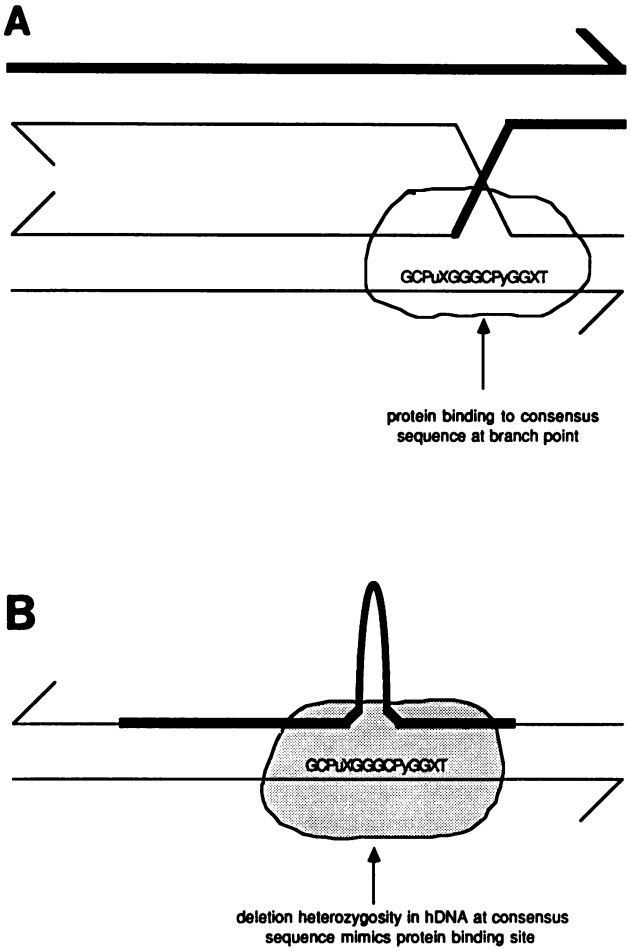

FIG. 5. Provision of a similar binding site by a deletion heterozygosity in heteroduplex DNA for a protein which normally recognizes a related sequence at a Holliday junction.

while two positions are specified only as purine or pyrimidine, and the remaining two bases are unspecified. This sequence and the relevant regions of the high PMS alleles are shown in Fig. 3. A score for the best alignment of each sequenced deletion allele with the consensus sequence is shown in Table 2. The score was computed at the best of the 15 possible alignments between the consensus sequence and the 28-bp which segment symmetrically spans the deletion. Identity with a specified base was given 1 point, and 0.5 point was scored for a match at the positions designated purine or pyrimidine in the consensus. No points were scored for the nonspecified positions. The maximum possible score is 10, though on average, a random 13-bp sequence should score 2.75. Since 15 alignments are surveyed for each sequence, most will produce a higher score (here, ranging between 4.5 and 6) than that predicted for a random 13-base sequence. Clearly, extensive homology to the PMS consensus sequence is not observed for any allele not displaying PMS.

DISCUSSION

This investigation was undertaken as a result of the then unexpected finding that the *ade8-18* mutation is a 38-bp deletion (16). Previous data concerning the meiotic behavior

of deletion mutations in yeast had not revealed a single PMS event, but the deletions analyzed were several hundred base pairs in length or frameshift mutations (6). Accordingly, we postulated that intermediate size deletions (tens of base pairs) might constitute a class of high PMS mutations. The data presented here clearly refute such hypotheses. All of the deletions which were introduced at the *Hpa*I site in the *ADE8* gene failed to display PMS. However, there is no general depression of PMS at the *Hpa*I site, since single base changes at this site can result in variable levels of PMS which are as high as 50% in some cases (J. H. White, R. W. Anderson, and S. Fogel, manuscript in preparation). Furthermore, there is no general elevation of PMS at the *Xho*I site, since several deletions at this site displayed no detectable PMS.

When the PMS frequencies for the *Xho*I deletions are compared with one another, the small numbers of events require that statistical aspects be considered. For example, in the null hypothesis that PMS for *Xho*I deletions is uniform, 18.4% of aberrant tetrads would be PMS. This hypothesis predicts 4.2 PMS events for *x3a*, 3.7 for *x1e*, and 4.2 for *x30a*, but none were observed. Thus, for a Poisson distribution of events, we reject the null hypothesis at the 98% level of confidence. Since *ade8-18* displays much higher PMS, we also analyzed the *Bal*31 deletions alone. When *ade8-18*, an allele of spontaneous origin (3), is disregarded and only *Bal*31-generated alleles are compared, the difference is less. Only 8.3% of aberrant tetrads would be PMS, which would predict 1.9 PMS events each for *x3a* and *x30a* and 1.7 for *x1e*. Sampling considerations place only an 82% level of confidence in stating that these events would have been detected. However, the same conservative null hypothesis predicts only 2.2 PMS events for *x1f*, compared with the 6 events which were observed. Completing the expectations for the conservative null hypothesis yields 1.3 predicted PMS events versus 2 which were observed for *x5l* and 1.4 predicted versus 2 which were observed for *x5d*. Thus, the statistical significance of differences in PMS frequencies among the alleles depends on the inclusion of *ade8-18* in the high PMS group.

A common sequence was identified spanning or adjacent to the deletion breakpoint in the nucleotide sequences of those alleles displaying PMS. This sequence is compared to a few selected sequence elements of general interest to recombination in Fig. 4. The "minisatellite" is a sequence first identified as the core for a repeated sequence found in a human myoglobin intron (8). Further analysis revealed that this minisatellite may constitute a hotspot for recombination at various loci (8, 10, 15). Recently, a repeat which was partially homologous to the minisatellite was identified in the *I-J* subregion in the mouse (10, 15), a known recombination hotspot. Finally, portions of the *E. coli chi* sequence (13) are related to these sequences.

There is no a priori basis to expect a relationship between sequences involved with elevated levels of crossing over and a sequence which confers high PMS frequency on a deletion mutation. One possibility is that it is the high-PMS character of these sequences which results in the hyperrecombination phenotype. It is equally possible that the PMS is a result of the recombinogenic activity of the sequences. That is, the elevated PMS observed most likely results from a failure to repair heteroduplex DNA containing a nonmatch resulting from the deletion heterozygosity. It is possible that such correction failure is due to specific binding of a protein to the base of the unmatched DNA loop (11). The consensus sequence for PMS would then describe the sequence specificity for the binding of this protein. The normal role for this protein could be to serve as an anchor for crossed strand structures in recombination. Since the hypothetical protein is presumably binding to a Holliday junction, the subsequent resolution of this junction allows for the association of a binding site with a recombination hotspot. Heteroduplex DNA with a nonmatched loop at the binding sequence should mimic one of the homologs involved in a recombination intermediate joined by a Holliday junction (Fig. 5). Thus, our hypothetical protein should bind to the base of the loop and prevent recognition of the nonhomology and subsequent degradation of the loop or incision of the deletion strand.

The deletions displaying elevated PMS do not also exhibit elevated aberrant tetrad frequencies. Thus, in our hypothesis, the recombination initiation sequence must be present on both homologs for interaction to begin. This argument assumes that the sequence required for binding is longer than that which we have described in the consensus sequence, since the consensus sequence is homozygous in *ade8-18* or *x5l* mutant heterozygotes. The events that we observed are then due to initiation events proximal to the sequences which were analyzed.

The PMS frequencies among aberrant tetrads for the *Bal*31-generated deletions which display PMS are considerably lower than that for *ade8-18*. This would again suggest that our consensus sequence does not completely describe the presumptive binding sequence.

In conclusion, a sequence has been described which contributes to PMS of deletion heterozygosities. Further investigation is warranted to elucidate any role that this sequence might play in other aspects of recombination. For example, the consensus sequence reported here can be transplaced to the vicinity of predetermined sites, and the effects on gene conversion, postmeiotic segregation, and outside marker recombination can be studied.

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