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Measurements of Excision Repair Tracts Formed during Meiotic Recombination in *Saccharomyces cerevisiae*

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During meiotic recombination in the yeast *Saccharomyces cerevisiae*, heteroduplexes are formed at a high frequency between *HIS4* genes located on homologous chromosomes. Using mutant alleles of the *HIS4* gene that result in poorly repaired mismatches in heteroduplex DNA, we find that heteroduplexes often span a distance of 1.8 kb. In addition, we show that about one-third of the repair tracts initiated at well-repaired mismatches extend 900 bp.

During meiosis in *Saccharomyces cerevisiae*, heterozygous alleles *A* and *a* usually segregate 2*A*:2*a* into the four spores of the ascus. The two most common types of aberrant segregation are gene conversion events (3*A*:1*a* or 1*A*:3*a* segregation) and postmeiotic segregation (PMS events). For a heterozygous auxotrophic mutation, PMS events are signalled by sectored colonies formed when the spore colonies grown on rich medium are replica plated to omission medium. For most mutant alleles in yeast cells, PMS events are much rarer than gene conversion events (median frequencies of 0.5 and 5% of unselected tetrads, respectively [12]). The most common types of PMS tetrads in *S. cerevisiae* are those with 2*A*:1*a*:1*A*/*a*-sectored colony or 1*A*:2*a*:1*A*/*a*-sectored colony (12, 28). Since comparable patterns of PMS were first detected in eight-spored fungi (in which meiotic divisions are followed by a single mitotic division), these types of segregation are described as 5*A*:3*a* or 3*A*:5*a* tetrads, respectively. In order to use a single system of nomenclature, we describe gene conversion events as 6+:2m or 2+:6m and normal Mendelian segregation as 4+:4m.

Sectored spore colonies reflect the presence of heteroduplex DNA with an unrepaired base mismatch (10). Since, in *S. cerevisiae*, tetrads with a single PMS event (5+:3m or 3+:5m) are usually much more common than tetrads with two PMS events (aberrant 4+:4m), most heteroduplexes are thought to be the result of the nonreciprocal transfer of a single strand of DNA from one allele to the other (12, 28). For example, if one strand of DNA is donated from the *A* allele to the *a* allele, one would expect to get a 5*A*:3*a* pattern of segregation.

Although all PMS events are thought to represent unrepaired mismatches in heteroduplexes, two different models of gene conversion have been proposed. In one class of models, the conversion events are the result of repair of mismatches in heteroduplex DNA (17, 25, 31). Such repair events may involve excision of the mismatch and surrounding sequences from one strand of the duplex followed by repair of the resulting gap, using the other strand as the template for DNA synthesis. In the original version of the double-strand break repair model (38), it was proposed that most conversion events reflect the repair of a double-stranded gap with sequences derived from the homologous chromatid. Several lines of evidence indicate that most meiotic conversion in *S. cerevisiae* is the result of mismatch repair. First, DNA mismatch repair mutants of *S. cerevisiae* that increase the frequency of PMS while decreasing the frequency of gene conversion have been isolated (42). Second, the frequency of PMS per aberrant segregation depends on the particular base pair mismatch (5, 21, 41).

The length of meiotic gene conversion tracts in *S. cerevisiae* has been estimated in several studies. In studies of coconversion events at the *ARG4* locus, Fogel and Mortimer (11) estimated that the typical gene conversion tract spanned a few hundred base pairs. Conversion tracts of greater genetic distance (sometimes greater than 14 centimorgans) were found by DiCaprio and Hastings (7) at the *SUP6* locus. In experiments in which the physical distance between markers was known, the length of an average conversion tract was estimated to be 1.5 to 3.7 kb (4, 19, 37).

The length of a gene conversion tract is influenced by two factors: the length of the heteroduplex region and the length of the excision repair tract. The length of the heteroduplex region can be examined by measuring the frequency of co-PMS events for markers with defined physical map positions. Although co-PMS events have been detected in several studies in *S. cerevisiae* (7, 12), these events are usually infrequent because most of the mutant alleles used in these studies showed little PMS. In addition, the physical distances separating the markers were not known for most combinations of alleles. One exception to this generalization is an analysis of coconversion and co-PMS in strains heterozygous for the *arg4-16* and *arg4-17* mutations, located 214 bp apart (41). Of about 3,000 tetrads, 21 showed co-PMS for these markers and 190 showed coconversion (12).

The amount of DNA excised and resynthesized during mismatch repair (an excision repair tract) has been investigated in most detail in bacteria (26). One method of measuring such tracts is to transform cells with heteroduplexes containing multiple mismatches. Using this procedure, Wagner and Meselson (39) showed that the average length of a repair tract in *Escherichia coli* was 3 kb. Transformation studies in *Streptococcus pneumoniae* suggested that repair tracts in this organism range from 5 to 10 kb (14, 24). A cocorrection study in *S. cerevisiae* suggests that repair tracts on plasmids transformed into vegetatively dividing haploids are usually shorter than 1 kb (2).

A method of determining the length of meiotic excision tracts is to examine the effects of alleles that lead to

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well-repaired mismatches (low-PMS alleles) on the PMS frequency of nearby alleles that lead to poorly repaired mismatches (high-PMS alleles). The rationale for this approach is that high-PMS alleles may represent mismatches that are poorly recognized by the mismatch repair system; if a mutant allele that leads to a well-recognized mismatch is inserted nearby, the excision repair tract initiated at this allele may sometimes include the poorly recognized mismatch, leading to a reduction in PMS and an elevation in gene conversion (12, 16, 32). This result has been obtained in a number of studies. For example, the PMS rate of *arg4-16* is reduced from 48% of the aberrant segregations to about 28% in the presence of *arg4-17* (upstream of *arg4-16*) or *arg4-19* (downstream of *arg4-16*) (13). Since the *arg4-16* and *arg4-17* mutations are 214 bp apart (41), this result indicates that the excision repair tract spans 200 bp about half of the time. The PMS rate of *his1-49* was reduced from 85% of the aberrant segregations of 18% in the presence of the heterozygous *his1-1S* allele (15); the *his1-1S* allele by itself shows no PMS. In both studies, although the PMS level of the high-PMS allele was reduced, the level of PMS for the low-PMS allele was not elevated.

In quantitative studies of the length of the excision repair as measured by the technique described in the preceding paragraph, it is important to determine how often heteroduplexes include both mismatches. If heteroduplexes include only one of the two mismatches, an excision tract derived from that mismatch would have no opportunity to affect the gene conversion frequency of the other allele. One method of determining the length of heteroduplex within a gene is to analyze the co-PMS frequencies of mutant alleles that have high levels of PMS. We chose to examine heteroduplex formation and mismatch repair at the *HIS4* locus for several reasons. First, in the strain background that we use, mutations at the *HIS4* locus have an extraordinarily high level of aberrant segregation (approximately 10-fold higher than normal) (5, 28). Second, most of the aberrant segregations at this locus have a heteroduplex intermediate (28). Third, the *HIS4* gene and many mutant derivatives have been sequenced (1, 8, 9, 29). Using strains that contain one or more of these sequenced mutations, we show that (i) many of the heteroduplexes at the *HIS4* locus span alleles located near the 5′ and 3′ ends of the gene (1.8 kb) and (ii) almost all excision repair tracts are longer than 26 bp and about one-third span interallelic distances of 900 bp.

MATERIALS AND METHODS

Plasmids and *E. coli* strains. Plasmids phis4-519 (B137), phis4-712 (B201), phis4-713 (B138), and phis4-Δ29 (B136) were gifts from B. Ruskin and G. Fink. These plasmids contain mutant *his4* genes on an *Eco*RI-*Bam*HI fragment inserted into *Eco*RI-*Bam*HI sites of YIp5. The *his4* mutations in these plasmids were sequenced previously (9) and are shown in Table 1. The *his4-3133* allele was constructed by inserting the self-annealed palindromic oligonucleotide (5′-CTAGTTACTGTATGTACATACAGTAA) into the *Xba*I site (located near the 3′ end of *HIS4*) of pMW2. The pMW2 plasmid contains a *Bgl*II fragment with the 3′ end of *HIS4* inserted into the *Bam*HI site of YIp5 (a gift of M. White). The plasmid resulting from the insertion of the palindrome into pMW2 is pPD6. The site of insertion was confirmed by DNA sequence analysis using methods described by Kraft et al. (20).

E. coli DK1 [Δ(*srl-recA*)*306 araD139* Δ(*ara leu*)*7697* Δ*lacX74 galU galK hsdR strA mcrA mcrB* (22)] was used to propagate plasmid pPD6. *E. coli* DB6507 [*hsd S20* (r_B^- m_B^-) *supE44 ara14* λ- *galK2 lacY1 proA2 rspL20 xyl-5 mtl-1 recA13 mcrA*(+) *mcrB*(−) *tyrS74*::Tn*5*Km^r *strA*] was used to propagate plasmids phis4-519, phis4-712, phis4-713, and phis4-Δ29.

TABLE 1. Related haploid yeast strains constructed by the two-step transplacement procedure

Strain name	Untransformed strain	Plasmid	Relevant genotype[a]	Position (type) of mutation[b]
PD7	AS4	phis4-519	*his4-519*	+493 (insertion of a G)
PD22	AS13	phis4-712	*his4-712*	+1396 (insertion of a G)
PD25	AS4	phis4-712	*his4-712*	+1396 (insertion of a G)
PD23	AS4	phis4-713	*his4-713*	+2270 (insertion of a G)
PD24	AS13	phis4-713	*his4-713*	+2270 (insertion of a G)
DNY47[c]	AS13	pDN22	*his4-IR9*	+467 (18-bp palindromic insertion)
PD21	AS13	phis4-Δ29	*his4-Δ29*	−87 to +938 (deletion)
PD68	AS4	phis4-Δ29	*his4-Δ29*	−87 to +938 (deletion)
PD100	DNY47	pPD6	*his4-IR9*	+467 (18-bp palindromic insertion)
			his4-3133	+2327 (26-bp palindromic insertion)

[a] Mutational changes made within the *HIS4* region.
[b] The +1 position represents the first base pair in the initiating ATG of *HIS4*.
[c] Strain constructed previously (27).

Yeast strains. All yeast strains used in this study were derivatives of AS4 (*MAT*α *trp1 arg4 tyr7 ade6 ura3 MAL2*) and AS13 (*MAT*a *leu2 ade6 ura3*) (36). The strains with various *his4* mutations were constructed by the two-step transplacement procedure (33, 43). For the transformation step, plasmids phis4-519, phis4-17, phis4-712, and phis4-713 were treated with *Spe*I to target the integration (30). Subsequently, Ura$^-$ derivatives of the transformed strains were selected using 5-fluoro-orotate (3) and screened for those derivatives that were also His$^-$. Table 1 summarizes the haploid strain constructions.

In the construction of yeast strains harboring two *his4* mutant alleles, complementation tests (described below) were used to determine the genotype of His$^-$ transformants. The resulting haploid strains were analyzed by Southern blotting to ensure that the two-step transplacement procedure removed the vector sequences. The resulting haploids were then mated to generate diploids as shown in Table 2.

Media and genetic techniques. Standard procedures and media were used (35). Cells were sporulated on plates (1%

TABLE 2. Construction of related diploid strains

Strain name	Strain construction	Relevant genotype[a]
PD107	AS13 × PD7	*HIS4/his4-519*
PD108	AS13 × PD25	*HIS4/his4-712*
PD109	AS13 × PD23	*HIS4/his4-713*
PD99[b]	PD98 × AS4	*his4-3133/HIS4*
DNY48[c]	DNY47 × AS4	*his4-IR9/HIS4*
PD93	DNY47 × PD7	*his4-IR9/his4-519*
PD94	DNY47 × PD23	*his4-IR9/his4-713*
PD95	DNY47 × PD25	*his4-IR9/his4-712*
PD101	PD100 × AS4	*his4-IR9 his4-3133/HIS4*

[a] Mutational changes within the *HIS4* region.
[b] Strain constructed previously (6).
[c] Strain constructed previously (27).

TABLE 3. Number of tetrads with aberrant segregations of *his4* mutant alleles

Strain	Mutation	Position	No. of tetrads								% PMS	% Conversion	% Aberrant segregations	PMS/aberrant segregation (%)
			6+:2m	2+:6m	5+:3m	3+:5m	Aberrant 4+:4m	Other PMS[a]	Other non-PMS[b]	Total				
DNY48[c]	*his4-IR9*	+467	15	15	50	44	8	6	1	379	28	8	37	78
PD107	*his4-519*	+493	54	50	0	0	0	0	10	285	0	40	40	0
PD108	*his4-712*	+1396	13	27	0	1	0	0	2	200	0.5	21	22	2
PD109	*his4-713*	+2270	18	25	1	0	0	0	1	199	0.5	22	23	2
PD99[d]	*his4-3133*	+2327	22	19	33	39	6	6	1	344	24	12	37	67
PD101	*his4-IR9*	+467	12	15	43	45	11	3	1	338	30	8	38	78
	his4-3133	+2327	14	7	41	34	13	4	0	338	27	6	33	81
PD93	*his4-IR9*	+467	56	34	9	10	0	2	3	362	6	26	31	18
	his4-519	+493	36	59	3	4	0	1	3	362	2	27	29	8
PD95	*his4-IR9*	+467	26	14	22	29	3	5	0	298	20	13	33	60
	his4-712	+1396	20	39	2	1	0	0	0	298	1	20	21	5
PD94	*his4-IR9*	+467	15	15	32	39	3	5	1	336	24	9	33	72
	his4-713	+2270	28	32	0	1	0	0	1	336	0.3	18	18	2

[a] Tetrads with more than one PMS event (other than an aberrant 4:4) or a conversion event and a PMS event.
[b] Tetrads with two conversion events (8+:0m or 0 +:8m).
[c] Reference 27.
[d] Data from reference 6.

potassium acetate, 0.1% yeast extract, 0.05% dextrose, 2% agar supplemented with adenine [6 μg/ml]) at 18°C (5). Tetrads were dissected on YPD plates (35) that were incubated at 32°C. The resulting spore colonies were replica plated to various types of omission media.

For strains that were heterozygous for two *his4* mutations (PD93, PD94, PD95, and PD101), complementation analysis was performed. This analysis is possible because the *HIS4* gene has three distinct regions (*HIS4A*, *HIS4B*, and *HIS4C*), each of which codes for a separate enzymatic activity in a trifunctional protein (18). Since the *his4-IR9* mutation is an in-frame insertion of a palindromic oligonucleotide in *HIS4A*, it is complemented by *his4-712*, *his4-713*, or *his4-280*, all of which represent *his4C* mutations. The *his4-Δ29* mutation is an in-frame deletion which is *his4A*, *his4B*, *HIS4C*. It complements *his4-712* and *his4-713* but does not complement *his4-IR9*. The *his4-519* mutation is a single-base-pair insertion in *HIS4A*, resulting in a mutant phenotype for all three *HIS4* enzymatic activities. Segregation of this allele was examined by using tester strains PD18 (*MATα SUF1 his4-Sal*) and PD19 (*MATα SUF1 his4-Sal*); *SUF1* is a dominant frameshift suppressor that suppresses *his4-519* (34). The complementation and suppression tests were done by mating replicas of the original dissection plate to lawns of tester strains. The lawns were made by spraying a stationary-phase culture of a tester strain to YPD plates with a V#1 Paasche airbrush. The plates were allowed to dry at 37°C for approximately 15 min, and the replica of the spore colonies was imprinted on the lawn. After 12 to 16 h at 32°C, the plate was replicated to a plate lacking histidine, and the replica was incubated overnight.

Statistical methods. Most of the statistical comparisons were done by using a contingency chi-square analysis; a chi-square value greater than 3.85 is significant at a level of $P < 0.05$. In comparisons in which one or more classes had fewer than five events, we used the StatXact program (Cytel Software Corp.) to determine P values by the Fisher exact test.

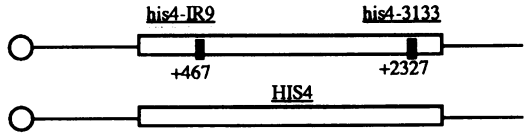
RESULTS

Level and extent of the heteroduplex intermediate at *HIS4*. High-PMS alleles in yeast cells can be constructed by the insertion of palindromic oligonucleotides (28). When such insertions are heterozygous, about 70% of the aberrant segregation events are PMS events. In previous studies, we found that strains heterozygous for palindromic insertions near the 5′ end of *HIS4* have a PMS frequency of about 26% and a gene conversion frequency of about 8% (28). A strain with a palindromic insertion at the 3′ end of *HIS4* (*his4-3133*) had similar frequencies of PMS (26%) and gene conversion (12%) (6). One interpretation of this result is that most of the heteroduplexes that include the 5′ region of *HIS4* also include the 3′ region, a distance of 1.8 kb. To test this possibility, we constructed a strain (PD101) that was heterozygous for two palindromic insertions, *his4-3133* (at +2327) and *his4-IR9* (at +467). The diploid was sporulated, and we examined the frequency of PMS and gene conversion events at both mutant sites (as described in Materials and Methods).

Of the 338 tetrads examined, 150 (44%) had an aberrant segregation for at least one of the two *his4* mutations (Tables 3 and 4). The frequencies of aberrant segregation for the two mutant alleles were similar, 38% for *his4-IR9* and 33% for *his4-3133*. These frequencies of aberrant segregation are similar to those observed for the same mutations in strains that were heterozygous for only one mutation (Table 3). Although there was a reduction in the level of gene conversion (from 12% to 6%) of *his4-3133* in PD101 relative to PD99, the levels of PMS and conversion of *his4-IR9* were similar in the presence (PD101) and absence (DNY48) of *his4-3133*. Thus, the two mutations in PD101 do not appear to interact in a way that grossly alters the pattern of recombination.

One common class of PMS tetrads (class 1; Fig. 1) was that in which both mutant alleles segregated 5+:3m (20 tetrads) or both segregated 3+:5m (20 tetrads) (Table 4). In 35 of the 40 tetrads, the same spore colony was sectored (reflecting PMS) for both mutant alleles; this result strongly suggests that most of the class 1 tetrads represent a single heteroduplex that spans both alleles, rather than two independent heteroduplexes; one example of a class 1 tetrad is shown in Fig. 1. In PD101, one chromosome contains the wild-type *HIS4* allele and the other contains both mutant alleles. In all 35 tetrads in which a single spore colony sectored for both mutant alleles, one sector had the *HIS4*

TABLE 4. Number of tetrads (and cosectoring patterns) in various classes of aberrant segregants for *his4* mutant alleles in PD101



his4-IR9 / his4-3133	5+:3m	3+:5m	6+:2m	2+:6m	Aberrant 4+:4m	Other[a]	4+:4m	Totals
5+:3m	20 (18)[b]	6 (3)	0	2	5 (5)	1	7	41
3+:5m	2 (1)	20 (17)	0	4	0	1 (1)	7	34
6+:2m	2	1	7	0	0	0	4	14
2+:6m	0	0	0	4	0	2	1	7
Aberrant 4+:4m	0	6 (4)	0	1	5 (9)	0	1	13
Other[b]	3 (2)	0	0	0	1 (2)	0	0	4
4+:4m	16	12	5	4	0	0	188[c]	225
Totals	43	45	12	15	11	4	208	338

[a] Includes 8+:0m, 0+:8m, ab6+:2m, ab2+:6m, 7+:1m, and 1+:7m tetrads and tetrads with three PMS events for one or more alleles.
[b] Each number without parentheses is the number of tetrads in each class. The number in parentheses indicates the number of spore colonies among these tetrads that were sectored in the same direction for both *his4-IR9* and *his4-3133*. For example, there were six tetrads that segregated 5+:3m for *his4-3133* and 3+:5m for *his4-IR9*. In three of six tetrads, the same spore colony sectored for both *his4-IR9* and *his4-3133*.
[c] There were five crossovers between the *his4* mutations in this class.

allele and the other had both mutant alleles. This result also supports the conclusion that these tetrads represent transfer of a single continuous strand of DNA from one chromosome to the other, without correction of either of the resulting two mismatches.

Another common class of aberrant segregation pattern (class 2; examples shown in Fig. 1) was that in which one mutant allele showed PMS (either 5+:3m or 3+:5m) and the other allele showed normal Mendelian segregation (4+:4m). In 28 tetrads, the *his4-IR9* allele had a single PMS event (16, 5+:3m; 12, 3+:5m tetrads), with the *his4-3133* allele segregating 4+:4m. In 14 tetrads, the *his4-3133* allele had a single PMS event (7, 5+:3m; 7, 3+:5m tetrads), with the *his4-IR9* allele segregating 4+:4m. Such tetrads could reflect heteroduplexes that involved only one of the two alleles and failure to repair the single mismatch (class 2a; Fig. 1). Alternatively, these tetrads could reflect heteroduplexes that span both alleles in which one of the resulting mismatches is repaired to restore 4+:4m segregation and the other mismatch is unrepaired (class 2b; Fig. 1).

A third common class (class 3) were tetrads in which both mutant alleles segregated 6+:2m (seven tetrads) or both segregated 2+:6m (four tetrads). Such tetrads could reflect mismatch repair events in a single heteroduplex spanning both alleles (Fig. 1). Alternatively, this class could reflect a conversion event that did not involve a heteroduplex intermediate (for example, gene conversion as the result of repair of a double-stranded gap [38]).

The three classes described above can be explained by formation of single asymmetric heteroduplexes in which the resulting mismatches are either repaired or left unrepaired. Most of the other classes of aberrant segregation involve more than one sector per tetrad and, therefore, presumably more than one heteroduplex. The frequency of these events is approximately that expected if the heteroduplexes are initiated independently. More specifically, if the frequency of tetrads with single PMS events at the *his4-IR9* allele is 26% (88 of 338), the expected frequency of tetrads with two PMS events at that site is about 0.26^2, or 7%. The expected number of tetrads with two PMS events at that site is, therefore, about 23 (0.07 × 338). We observed 14 such tetrads (Table 3).

The most common class of tetrads with two PMS events (class 4) were those in which one allele had an aberrant 4:4 segregation (1 His^+ spore colony:2 sectored His^+/His^- colonies:1 His^- colony) and the other segregated 5+:3m or 3+:5m, or tetrads in which both alleles had the aberrant 4:4 segregation pattern (Table 4). The first of these classes could

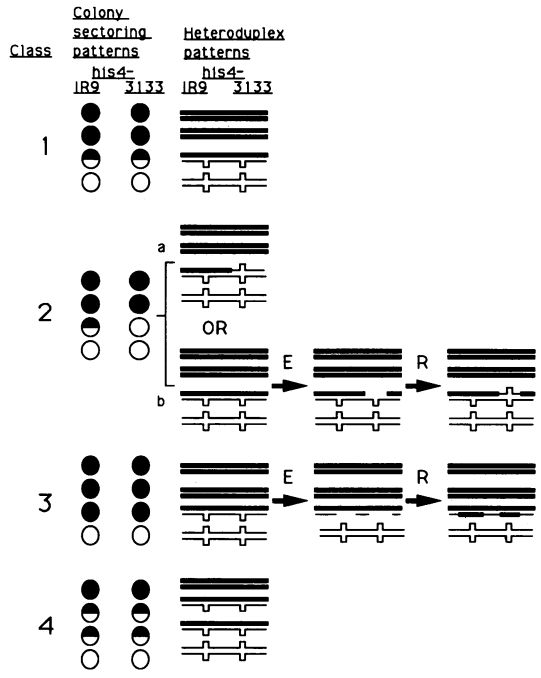


FIG. 1. Examples of colony sectoring patterns and interpretive heteroduplex patterns for various classes of aberrant segregants at the *HIS4* locus in the diploid strain PD101. In PD101, one chromosome contained a wild-type *HIS4* gene and the other contained two palindromic insertions (*his4-IR9* near the 5′ end of the gene and *his4-3133* near the 3′ end of the gene). The tetrads were dissected, and spores were allowed to form colonies on plates containing a rich growth medium. The resulting colonies were mated to various tester strains and replica plated to medium lacking histidine (as described in Materials and Methods) in order to determine the segregation pattern of each mutant allele separately. Black circles indicate His^+ colonies, and white circles indicate His^- colonies. Sectored circles reflect PMS events. Thus, in the example of a class 1 aberrant segregation shown (classes are defined in the text), for both alleles, two of the spore colonies have only the wild-type allele and one has only the mutant allele. In this example, the same spore colony sectors for both mutant alleles. The simplest interpretation of this sectoring pattern is that one of the wild-type *HIS4* genes donated a single DNA strand to a mutant gene; the resulting heteroduplex spans both mutant alleles. In the heteroduplex diagrams, the wild-type and mutant DNA strands are indicated by thick and thin lines, respectively. The mutant substitutions are drawn as hairpins, since palindromic insertions would be expected to form such structures in heteroduplexes (28). The class 2 sectored colonies could be formed in two different ways: a heteroduplex covering only one allele and failure to repair the resulting mismatch (class 2a), or a heteroduplex covering both mutations, followed by correction of only one of the mismatches (class 2b). Step E represents the excision of the mismatch, and step R shows repair of the resulting gap. Class 3 and class 4 tetrads represent gene conversion events and aberrant 4:4 segregation events, respectively.

reflect formation of two heteroduplexes spanning both mutant alleles, followed by correction of one of the mismatches. Alternatively, this class could reflect independent formation of two heteroduplexes, one involving only one of the mutant alleles and one spanning both. There is an interesting asymmetry in this class; all tetrads with a single PMS event at *his4-IR9* and two PMS events at *his4-3133* represented 3+:5m tetrads at *his4-IR9*, whereas the comparable class of tetrads with a single event at *his4-3133* had only 5+:3m tetrads. One possible explanation for this finding is that mismatches involving the *his4-IR9* mismatch are more frequently repaired to generate a mutant gene than are mismatches involving the *his4-3133* hairpin. A slight bias of this sort is seen in the ratios of 6:2/2:6 tetrads in PD101, 12/15 for *his4-IR9*, and 14/7 for *his4-3133* (Table 3).

The class of tetrads in which both alleles showed the aberrant 4:4 segregation pattern is likely to represent independent formation of two heteroduplexes, each spanning both mutations (Fig. 1). In 14 of the 16 class 4 tetrads, the sectoring patterns were consistent with the formation of one or more heteroduplexes that span both mutant alleles (Table 4).

In summary, about half of the spore colonies that sectored for the *his4-IR9* allele cosectored at *his4-3133*; similarly, about half of the spore colonies that sectored for the *his4-3133* allele cosectored at *his4-IR9*. These results indicate that approximately half of the heteroduplexes initiated at the *HIS4* locus span both mismatches, a distance of 1.8 kb. This estimate is minimal because some tetrads in classes 2 and 3 may also represent heteroduplexes that span both mutant alleles.

Length of the meiotic excision repair tract. As discussed in the introduction, in a number of systems, it has been shown that the repair of a mismatch involves a local excision of DNA flanking the mismatch. One method of determining the size of this excision tract is to examine the effect of mutant alleles that lead to well-repaired mismatches (low-PMS alleles) on the segregation of closely linked alleles that lead to poorly repaired mismatches (high-PMS alleles). If the excision tract initiated from the low-PMS allele includes the high-PMS allele, then the level of PMS for the high-PMS allele should be reduced. The rationale for this type of analysis is shown in Fig. 2.

As shown in Table 3, a strain that is heterozygous for the high-PMS allele *his4-IR9* (at +467) by itself shows 28% PMS and 8% gene conversion per unselected tetrad (27). We made three strains heterozygous for *his4-IR9* and one of three mutant alleles that contain the insertion of a single G at different positions in the gene (*his4-519* [at +493], *his4-712* [at +1396], and *his4-713* [at +2270]). As shown in Table 3, these single-base insertions (when alone) show little or no PMS (less than 2% of the aberrant segregations), indicating that they lead to well-repaired mismatches in meiotic heteroduplexes. The aberrant segregation patterns of *his4-IR9* in the presence of each of these alleles are shown in Tables 3, 5, 6, and 7. It should be noted that, unlike the PD101 diploid, in the three diploids used in this analysis, one chromosome had the *his4-IR9* mutation and the other had the low-PMS *his4* mutation.

The *his4-519* mutation, located 26 bp from *his4-IR9*, reduces the PMS per tetrad of *his4-IR9* about fivefold (from 28% to 6% [$\chi^2 = 65$]). This decrease is compensated for by an increase in the rate of gene conversion of *his4-IR9* from 8% to 26% of the total tetrads ($\chi^2 = 40$). Most of *his4-IR9* gene conversions (97% of the 6+:2m and 2+:6m segregations) were coconversions with *his4-519* (Table 5). The coconversions (6+:2m and 2+:6m events only) between *his4-IR9* and *his4-519* represented 24% of the total tetrads. The dramatic reduction in PMS for *his4-IR9* and the concomitant increase in gene conversion represent the effects expected if the excision tract initiated at the well-repaired *his4-519* mismatch almost always includes the *his4-IR9* mismatch.

The allele *his4-712*, located 929 bp from *his4-IR9*, decreased the PMS of *his4-IR9* from 28% to 20% of the total tetrads ($\chi^2 = 6.3$) and increased the conversion rate of

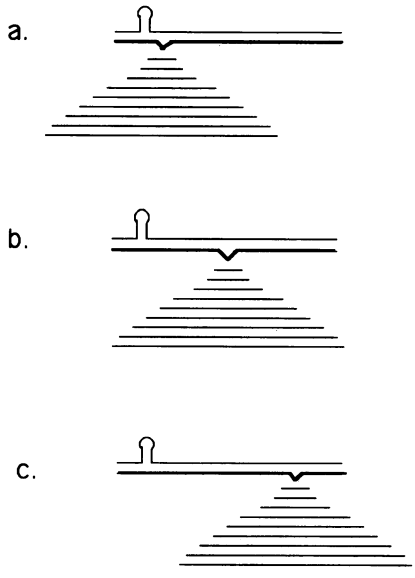


FIG. 2. Effects of a well-repaired mismatch on neighboring poorly repaired mismatches. Shown are heteroduplexes formed between two mutant *his4* genes, one containing the *his4-IR9* mutation (resulting in a poorly repaired mismatch) and one containing a well-repaired mismatch. The positions of the mismatches are indicated by the hairpin (*his4-IR9*) or triangles (other mismatches). The horizontal lines emanating from each well-repaired mismatch represent regions of DNA that are excised during excision of the mismatch. We show these excision tracts as occurring bidirectionally from the mismatch, although there are no data supporting this pattern. In addition, the distribution of tract lengths is not known. If the distribution of tract lengths is the same from each mismatch, then one would expect that an excision repair tract initiating from the well-repaired mismatch would affect the segregation of the poorly repaired mismatch in a distance-dependent manner. The figure is drawn to reflect the approximate distances between *his4-IR9* and *his4-519* (a), *his4-IR9* and *his4-712* (b), and *his4-IR9* and *his4-713* (c).

his4-IR9 from 8% to 13% of the total tetrads ($\chi^2 = 4.3$). Most of *his4-IR9* gene conversions (63% of the 6+:2m and 2+:6m segregations) were coconversions with *his4-712* (Table 6). The coconversions (6+:2m and 2+:6m events only) between *his4-IR9* and *his4-712* represented 8% of the total tetrads. Since the PMS level of *his4-IR9* was lowered by about one-third by the presence of *his4-712*, we suggest that approximately one-third of the excision tracts initiated at *his4-712* include *his4-IR9*. This estimate is a minimal one, since not all of the heteroduplexes that include the *his4-IR9* mismatch will also include *his4-712*.

The allele *his4-713*, located 1,803 bp from *his4-IR9*, had no significant effect on the segregation of *his4-IR9* (Table 7). In the presence of *his4-713*, PMS per tetrad for *his4-IR9* remained high at 24% (a rate statistically not different from 28% PMS per tetrad for *his4-IR9* alone [$\chi^2 = 2.0$]). Although *his4-713* also had no significant effect on gene conversion of *his4-IR9* ($\chi^2 = 0.13$), the two alleles still showed some coconversion (43% of all the 6+:2m and 2+:6m events at *his4-IR9* were coconversions with *his4-713*). The coconversions (6+:2m and 2+:6m events only) between *his4-IR9* and *his4-713* represented 3.9% of the total tetrads. These results indicate that excision tracts initiated at *his4-713* usually do not include *his4-IR9*.

Induction of PMS at a well-repaired mismatch by a closely linked poorly repaired mismatch. Although previous studies (12, 15, 23) showed that a well-repaired allele can reduce the PMS of a nearby poorly repaired allele, the poorly repaired allele was not observed to increase the frequency of PMS for the well-repaired alleles. In contrast, we found that *his4-IR9* significantly increased the PMS rate of *his4-519* (located 26 bp away) from 0% to 2% of total tetrads (Fisher exact test; $P = 0.009$). As shown in Table 5, each of the PMS events at *his4-519* had a co-PMS event at *his4-IR9*. No statistically significant increase in PMS was measured in the presence of *his4-IR9* for the two alleles *his4-712* (929 bp downstream of *his4-IR9*) and *his4-713* (1,803 bp downstream of *his4-IR9*) (Fisher exact test; $P = 0.34$ and 0.47, respectively).

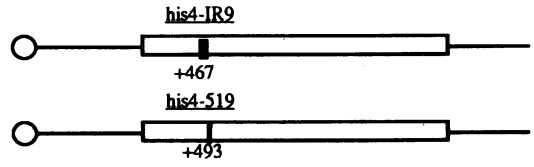
DISCUSSION

From our meiotic analysis of mutant *his4* alleles separated by various known physical distances, we conclude that (i) meiotic heteroduplexes often span most of the *HIS4* gene (a distance of 1.8 kb), (ii) excision-repair tracts almost always extend 26 bp and about one-third of the time extend 900 bp, and (iii) certain high-PMS alleles can elevate the frequency of PMS for very closely linked low-PMS alleles. Each of these conclusions will be discussed further below.

As shown in Table 4, approximately half of the PMS events at *his4-IR9* were associated with a PMS event at *his4-3133*. The simplest interpretation of this observation is that the co-PMS pattern reflects a continuous asymmetric heteroduplex spanning the 1.8-kb distance between the mutant alleles. As indicated in Fig. 1, those tetrads with PMS events involving only one of the two mutant alleles could reflect either heteroduplexes that cover only one allele or repair of one of the two mismatches. The observation that the level of PMS was similar for *his4-IR9* at the 5′ end of the gene and *his4-3133* at the 3′ end of the gene is consistent with previous conclusions based on the analysis of strains containing single *his4* mutations (6). It should be emphasized that the high level of PMS observed for *his4-3133* does not reflect the ability of palindromic mutations to initiate heteroduplexes. The levels of aberrant segregation of *his4-IR9* are identical in the presence and absence of *his4-3133*. In addition, the level of aberrant segregation for *his4-3133* is identical to that observed for the high-PMS *his4-200* allele, a base substitution at the 3′ end of *HIS4* (6).

The well-repaired alleles *his4-519*, *his4-712*, and *his4-713* are located within this 1.8-kb region known to be frequently spanned by meiotic heteroduplexes. Thus, when *his4-IR9* is involved in a meiotic heteroduplex, this heteroduplex should span the site of each of these alleles more than half of the time. We found that the presence of the heterozygous *his4-519* allele (at +493) greatly decreased the PMS and increased the gene conversion rate of *his4-IR9* (at +467). This observation suggests that the repair of *his4-519* mismatches in a heteroduplex stimulates the repair of *his4-IR9* mismatches in the same heteroduplex. Since the reduction in the frequency of PMS for *his4-IR9* (from 28% to 6% of the total tetrads) is nearly balanced by the increase in gene conversion (from 8% to 26%), we conclude the most of the repair events induced by *his4-519* represent conversion-type repair rather than restoration-type repair. This conclusion supports previous studies indicating that most repair events near the 5′ end of *HIS4* represent conversion-type repair (5). In addition, these results indicate that (i) mismatches resulting from heterozygous palindromic insertions are not well recognized by the meiotic mismatch repair system and (ii)

TABLE 5. Number of tetrads (and cosectoring patterns) in various classes of aberrant segregants for *his4* mutant alleles in PD93[a]



his4-519 \ his4-IR9	5+:3m	3+:5m	6+:2m	2+:6m	Aberrant 4+:4m	Other	4+:4m	Totals
5+:3m	0	3 (3)	0	0	0	0	0	3
3+:5m	4 (4)	0	0	0	0	0	0	4
6+:2m	0	4	0	31	0	1	0	36
2+:6m	2	0	56	0	0	0	1	59
Aberrant 4+:4m	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	4(1)	0	4
4+:4m	3	3	0	3	0	0	247[b]	256
Totals	9	10	56	34	0	5	248	362

[a] See Table 4, footnotes *a* and *b*.
[b] There were no crossovers between the *his4* mutant alleles in this class.

such mismatches can be repaired by excision tracts initiated elsewhere.

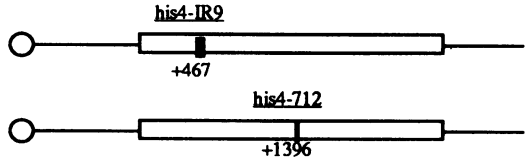
In the presence of *his4-712*, the frequency of PMS of *his4-IR9* drops by one-third, with a comparable elevation in gene conversion, indicating that repair tracts involved in the repair of mismatches at *his4-712* span the 929 bp between the two alleles about one-third of the time. This result also shows that mismatches are not repaired in the same way in every tetrad. No effect of the *his4-713* mutation on the PMS frequency of *his4-IR9* was observed, indicating that excision repair tracts usually do not span a distance of 1.8 kb. It should be pointed out, however, that not all of the heteroduplexes that involve *his4-IR9* also include the 3′ end of the gene; as discussed above, we estimate that at least half of the heteroduplexes span the 5′ and 3′ alleles. PMS events at *his4-IR9* that do not involve a heteroduplex at *his4-713* would not be expected to be affected by the *his4-713* mutation. Consequently, we cannot exclude the possibility that some fraction (one-third or less) of the excision repair events initiated at the *his4-713* mismatch extend to *his4-IR9*.

The frequency of repair tracts reaching *his4-IR9* might be influenced by the nature of sequences or chromosomal structures between *his4-713* and *his4-IR9*, as well as by the physical distance between the alleles. A sequence that inhibits meiotic mismatch repair has been reported (40), although this sequence is not present in the *HIS4* gene. Although it is not possible to calculate accurately an average tract length on the basis of our data (since we do not accurately know the distribution of tract lengths and we do not know whether the excision events occur unidirectionally or bidirectionally from the mismatch), if we assume a normal distribution of tract lengths, we estimate that the average excision repair tract length is propagated 500 to 800 bp from the initiating mismatch (either uni- or bidirectionally).

Two additional caveats should be mentioned. First, it is possible that the mismatch signals the excision event, but the excision tract does not initiate at the mismatch. Second, the plasmid-borne *HIS4* gene used in our study to introduce mutations was not derived from our yeast strain; therefore, additional hidden heterozygous markers may be present in our strains. Although this possibility cannot be excluded, it is unlikely for several reasons. First, such hidden heterozygous markers would be expected to obscure the distance-dependent effects of *his4-519*, *his4-712*, and *his4-713* on the PMS frequency of *his4-IR9*. Second, the levels of PMS for both palindromic insertions in the strain PD101 were both very high, about 80% of the total aberrant segregants; hidden heterozygosity should lower the frequency of PMS relative to gene conversion.

Our results agree with those of Fogel et al. (12, 13), indicating that the meiotic mismatch repair tracts in *S. cerevisiae* are frequently more than 214 bp in length; this agreement suggests that the unusually high rate of aberrant segregation of *HIS4* seen in our genetic background does not

TABLE 6. Number of tetrads (and cosectoring patterns) in various classes of aberrant segregants for *his4* mutant alleles in PD95[a]



his4-712 \ his4-IR9	5+:3m	3+:5m	6+:2m	2+:6m	Aberrant 4+:4m	Other	4+:4m	Totals
5+:3m	0	1 (1)	0	0	0	0	1	2
3+:5m	0	0	1	0	0	0	0	1
6+:2m	0	6	0	9	0	0	5	20
2+:6m	9	2	16	1	1	0	10	39
Aberrant 4+:4m	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0
4+:4m	13	20	9	4	2	5	183[b]	236
Totals	22	29	26	14	3	5	199	298

[a] See Table 4, footnotes *a* and *b*.
[b] There were six crossovers in this class between the *his4* mutant alleles.

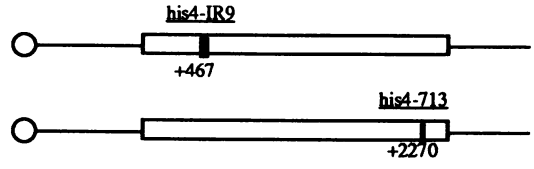
reflect any gross alteration in the length of excision repair tracts. The results also agree with the mitotic study of Bishop and Kolodner (2) which indicates that mismatches separated by 0.9 kb are sometimes not involved in the same repair tract during mitotic repair. Since the meiotic and mitotic repair systems in *S. cerevisiae* share the same specificity toward individual base mismatches (2, 5, 21), these two systems are likely to be very similar or identical. Meiotic repair tracts in *S. cerevisiae* are similar in length to those in *E. coli*, which have an average length of 3 kb (39), and are shorter than those in *S. pneumoniae*, which range from 5 to 10 kb (14, 24).

As discussed previously, if all gene conversion events reflect mismatch repair in heteroduplex DNA, the length and continuity of conversion tracts will be a function of the lengths of the heteroduplex and the excision repair tract. In *S. cerevisiae*, the average meiotic gene conversion tract length has been estimated to be 1.5 kb at the *MAT* locus (4), 2.3 kb at *URA3* (19), and 3.7 kb in the *LEU2-CEN3* region (37). In one study (37), 20% of all conversion tracts measured were more than 5 kb. In all these studies, the conversion tracts were almost always continuous (sites showing gene conversion were not separated by sites showing normal Mendelian segregation). Although our studies indicate that the length of the heteroduplexes may be sufficient to account for most of the conversion data, it is not clear that our data explain the continuity of very long (greater than 5 kb) conversion tracts. Possible explanations of this discrepancy are as follows: (i) long meiotic conversion tracts might reflect some mechanism other than heteroduplex formation and repair (such as the repair of double-stranded gaps with the sequences from a homologous chromosome [38]), (ii) one strand of a heteroduplex may be tagged for excision by a mechanism analogous to methyl-directed repair in *E. coli*, and (iii) the lengths of repair tracts are different in different regions of the genome.

Our experiments showed that the PMS rate of *his4-IR9* was severely reduced by the presence of *his4-519* (an allele that rarely shows PMS). In addition, the PMS rate of *his4-519* is increased by the presence of *his4-IR9*. The effects these alleles have on each other are not of equal magnitude. The decrease in PMS of *his4-IR9* involves about 20% of the total tetrads, whereas the increase in PMS of *his4-519* involves only 2% of the tetrads. Although decreases in PMS by the presence of another allele have been seen previously (12, 15, 23), the induction of PMS of one allele by the presence of another allele has not yet been reported.

One of the differences between this study and previous studies is that *his4-IR9* is a palindromic insertion. Evidence indicates that palindromic insertions show high PMS as a result of their ability to undergo intrastrand base pairing resulting in a hairpin configuration (28). Although many of

TABLE 7. Number of tetrads (and cosectoring patterns) in various classes of aberrant segregants for *his4* mutant alleles in PD94[a]



his4-713 \ his4-IR9	5+:3m	3+:5m	6+:2m	2+:6m	Aberrant 4+:4m	Other	4+:4m	Totals
5+:3m	0	0	0	0	0	0	0	0
3+:5m	0	0	0	0	0	0	1	1
6+:2m	3	8	2	7	0	0	8	28
2+:6m	16	3	6	0	0	0	7	32
Aberrant 4+:4m	0	0	0	0	0	0	0	0
Other	1	0	0	0	0	0	0	1
4+:4m	12	28	7	8	3	6	210[b]	274
Totals	32	39	15	15	3	6	226	336

[a] See Table 4, footnotes *a* and *b*.
[b] There were three crossovers between the *his4* mutant alleles in this class.

the *his4-IR9* hairpins are repaired in the presence of *his4-519*, 38% of the hairpins which escape repair are involved with a co-PMS event at *his4-519*. All of the PMS events at *his4-519* are co-PMS events with *his4-IR9*. The distance separating these alleles is 26 bp; no induction of PMS was seen for alleles at a further distance. One interpretation of this result is that there are proteins bound to the hairpin that inhibit its repair or recognition and these proteins can sometimes inhibit the repair of very closely linked mutations. Since previous studies did not investigate mutant alleles that were extremely closely linked, it is also possible that the reciprocal effects that we have detected are due to the short distance between the mismatches rather than the type of high-PMS allele.

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REFERENCES

1. **Arndt, K. T., C. Styles, and G. R. Fink.** 1987. Multiple global regulators control *HIS4* transcription in yeast. Science **237**:874–880.
2. **Bishop, D. K., and R. D. Kolodner.** 1986. Repair of heteroduplex plasmid DNA after transformation into *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:3401–3409.
3. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
4. **Borts, R. H., and J. E. Haber.** 1989. Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae*. Genetics **123**:69–80.
5. **Detloff, P. D., J. Sieber, and T. D. Petes.** 1991. Repair of specific base pair mismatches formed during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:737–745.
6. **Detloff, P. D., M. A. White, and T. D. Petes.** Unpublished data.
7. **DiCaprio, L., and P. J. Hastings.** 1976. Gene conversion and intragenic recombination at the *SUP6* locus and the surrounding region in *Saccharomyces cerevisiae*. Genetics **84**:697–721.
8. **Donahue, T. F., and A. M. Cigan.** 1988. Genetic selection for mutations that reduce or abolish ribosomal recognition of the *HIS4* translational initiator region. Mol. Cell. Biol. **8**:2955–2963.

9. **Donahue, T. F., P. J. Farabaugh, and G. R. Fink.** 1982. The nucleotide sequence of the *HIS4* region of yeast. Gene **18:**47–59.
10. **Esposito, M. S.** 1971. Post-meiotic segregation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **111:**297–299.
11. **Fogel, S., and R. K. Mortimer.** 1969. Informational transfer in meiotic gene conversion. Proc. Natl. Acad. Sci. USA **62:**96–103.
12. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1981. Mechanisms of meiotic gene conversion, or "wanderings on a foreign strand," p. 289–339. *In* J. Strathern, E. W. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Fogel, S., R. K. Mortimer, K. Lusnak, and F. Tavares.** 1979. Meiotic gene conversion—a signal of the basic recombination events in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.
14. **Guild, W. R., and N. B. Shoemaker.** 1976. Mismatch correction in pneumococcal transformation: donor length and *hex*-dependent marker efficiency. J. Bacteriol. **125:**125–135.
15. **Hastings, P. J.** 1984. Measurement of restoration and conversion: its meaning for the mismatch repair hypothesis of conversion. Cold Spring Harbor Symp. Quant. Biol. **49:**49–53.
16. **Hastings, P. J.** 1988. Conversion events in fungi, p. 397–428. *In* R. Kucherlapati and G. Smith (ed.), Meiosis. American Society for Microbiology, Washington, D.C.
17. **Holliday, R.** 1964. A mechanism for gene conversion in fungi. Genet. Res. **5:**282–304.
18. **Jones, E. W., and G. R. Fink.** 1981. Regulation of amino acids and nucleotide biosynthesis in yeast, p. 181–299. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Judd, S. R., and T. D. Petes.** 1988. Physical lengths of meiotic and mitotic gene conversion tracts in *Saccharomyces cerevisiae*. Genetics **118:**401–410.
20. **Kraft, R., J. Tardif, K. S. Krauter, and L. A. Leinwand.** 1988. Using mini-prep plasmid DNA for sequencing double-stranded templates with Sequenase. Bio/Technology **6:**544–545.
21. **Kramer, B., W. Kramer, M. S. Williamson, and S. Fogel.** 1989. Heteroduplex DNA correction in *Saccharomyces cerevisiae* is mismatch specific and requires functional *PMS* genes. Mol. Cell. Biol. **9:**4432–4440.
22. **Kurnit, D. M.** 1989. *Escherichia coli recA* deletion strains that are highly competent for transformation and for *in vivo* phage packaging. Gene **82:**313–315.
23. **Leblon, G., and J.-L. Rossignol.** 1973. Mechanism of gene conversion in *Ascobolus immersus*. III. The interactions of heteroalleles in the conversion process. Mol. Gen. Genet. **122:**165–182.
24. **Mejean, V., and J. P. Claverys.** 1984. Effect of mismatched base pairs on the fate of donor DNA in transformation of *Streptococcus pneumoniae*. Mol. Gen. Genet. **197:**467–471.
25. **Meselson, M., and C. Radding.** 1975. A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:**358–361.
26. **Modrich, P.** 1987. DNA mismatch correction. Annu. Rev. Biochem. **56:**435–466.
27. **Nag, D. K., and T. D. Petes.** 1991. Seven base-pair inverted repeats in DNA form stable hairpins *in vivo* in *S. cerevisiae*. Genetics **129:**669–673.
28. **Nag, D. K., M. A. White, and T. D. Petes.** 1989. Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature (London) **340:**318–320.
29. **Nagawa, F., and G. R. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation sites at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82:**8557–8561.
30. **Orr-Weaver, T. L., J. W. Szostak, and R. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:**6354–6358.
31. **Radding, C. M.** 1982. Homologous pairing and strand exchange in genetic recombination. Annu. Rev. Genet. **16:**405–437.
32. **Rossignol, J.-L., A. Nicolas, H. Hamza, and A. Kalogeropoulos.** 1988. Recombination and gene conversion in Ascobolus, p. 23–72. *In* K. B. Low (ed.), The recombination of genetic material. Academic Press, New York.
33. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76:**4951–4955.
34. **Sherman, F.** 1981. Suppression in the yeast *Saccharomyces cerevisiae*, p. 463–486. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Stapleton, A., and T. D. Petes.** 1991. The Tn3 β-lactamase gene acts as a hotspot for meiotic recombination in yeast. Genetics **127:**39–51.
37. **Symington, L. S., and T. D. Petes.** 1988. Expansions and contractions of the genetic map relative to the physical map of yeast chromosome III. Mol. Cell. Biol. **8:**595–604.
38. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33:**25–35.
39. **Wagner, R., and M. Meselson.** 1976. Repair tracts in mismatched DNA heteroduplexes. Proc. Natl. Acad. Sci. USA **73:**4135–4139.
40. **White, J. H., J. F. DiMartino, R. W. Anderson, K. Lusnak, D. Hilbert, and S. Fogel.** 1988. A DNA sequence conferring high postmeiotic segregation frequency to heterozygous deletions in *Saccharomyces cerevisiae* is related to sequences associated with eucaryotic recombination hotspots. Mol. Cell. Biol. **8:**1253–1258.
41. **White, J. H., K. Lusnak, and S. Fogel.** 1985. Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. Nature (London) **315:**350–352.
42. **Williamson, M. S., J. C. Game, and S. Fogel.** 1985. Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I. Isolation and characterization of *pms1-1* and *pms1-2*. Genetics **110:**609–646.
43. **Winston, F., F. Chumley, and G. R. Fink.** 1983. Eviction and transplacement of mutant genes in yeast. Methods Enzymol. **101:**211–228.