Conversion-Type and Restoration-Type Repair of DNA Mismatches Formed During Meiotic Recombination in *Saccharomyces cerevisiae*

David T. Kirkpatrick, Margaret Dominska and Thomas D. Petes

Department of Biology, Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280

Manuscript received February 5, 1998
Accepted for publication April 16, 1998

ABSTRACT

Meiotic recombination in yeast is associated with heteroduplex formation. Heteroduplexes formed between nonidentical DNA strands contain DNA mismatches, and most DNA mismatches in wild-type strains are efficiently corrected. Although some patterns of mismatch correction result in non-Mendelian segregation of the heterozygous marker (gene conversion), one predicted pattern of correction (restoration-type repair) results in normal Mendelian segregation. Using a yeast strain in which a marker leading to a well-repaired mismatch is flanked by markers that lead to poorly repaired mismatches, we present direct evidence for restoration-type repair in yeast. In addition, we find that the frequency of tetrads with conversion-type repair is higher for a marker at the 5′ end of the *HIS4* gene than for a marker in the middle of the gene. These results suggest that the ratio of conversion-type to restoration-type repair may be important in generating gradients of gene conversion (polarity gradients).

IN the yeast *Saccharomyces cerevisiae*, both reciprocal (crossovers) and nonreciprocal (gene conversions) recombination events are associated with heteroduplexes (reviewed by Petes *et al.* 1991). Heteroduplexes are regions of DNA composed of DNA strands derived from two different chromosomes. In one model of recombination, these heteroduplexes are formed as a consequence of processing of the double-strand DNA break that initiates recombination (Szostak *et al.* 1983; Sun *et al.* 1989, 1991; Stahl 1996). Recombination events at the *HIS4* locus (Figure 1) are initiated by a double-strand break that occurs upstream of *HIS4*, followed by 5′ to 3′ degradation of the broken ends (Nag and Petes 1993; Porter *et al.* 1993; Fan *et al.* 1995).

If the two interacting chromosomes contain sequence differences in the heteroduplex region, one or more DNA mismatches will be generated (Figure 2). Repair of the resulting mismatch to the genotype of the donor allele (conversion-type repair) results in a gene conversion event, whereas repair to the genotype of the recipient (restoration-type repair) results in normal Mendelian segregation. Failure to repair the mismatch will generate a tetrad with postmeiotic segregation (PMS event), detected as a spore colony with sectors of two genotypes. Although gene conversion events in yeast are usually described as 3:1 or 1:3 segregation and normal Mendelian segregation is described as 2:2, we will use the nomenclature developed for eight-spored fungi because this nomenclature is better suited to describing tetrads with postmeiotic segregation. For tetrads derived from a strain heterozygous for alleles *A* and *a*, we will use the following nomenclature: normal 4:4 (2*A*:2*a* spore colonies), 6:2 (3*A*:1*a* spore colonies), 2:6 (1*A*:3*a* spore colonies), 5:3 (2*A*:1*a*:1 sectored *A*/*a* spore colonies), 3:5 (1*A*:2*a*:1 sectored *A*/*a* spore colonies), and aberrant 4:4 (1*A*:1*a*:2 sectored *A*/*a* spore colonies) tetrads. Aberrant segregation tetrads represent any pattern other than normal 4:4 segregation. The level of aberrant segregation at a particular locus reflects the frequency of heteroduplex formation at that site (Petes *et al.* 1991).

Because restoration-type repair does not result in aberrant segregation, it has been difficult to obtain direct evidence for this type of repair in yeast, although indirect arguments for its existence have been presented (Hastings 1984; Detloff *et al.* 1992). In contrast, restoration-type repair was directly demonstrated in the fungus *A. immersus* (Hastings *et al.* 1980). These workers constructed a strain that was heterozygous for four mutations in the *b2* locus. The two flanking markers, when located in a heteroduplex, resulted in poorly repaired mismatches and, therefore, high frequencies of PMS. The middle markers, when located in a heteroduplex, generated well-repaired mismatches (high gene conversion, low PMS). Hastings *et al.* (1980) examined the segregation pattern of the middle markers in octads in which the flanking markers had a PMS segregation pattern consistent with a single heteroduplex that included both flanking markers and the middle markers. They found that conversion-type and restoration-type repair occurred with similar frequencies. As described below, we used a similar procedure to prove the existence of restoration-type repair in yeast.

Corresponding author: Thomas D. Petes, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.
E-mail: tompetes@email.unc.edu

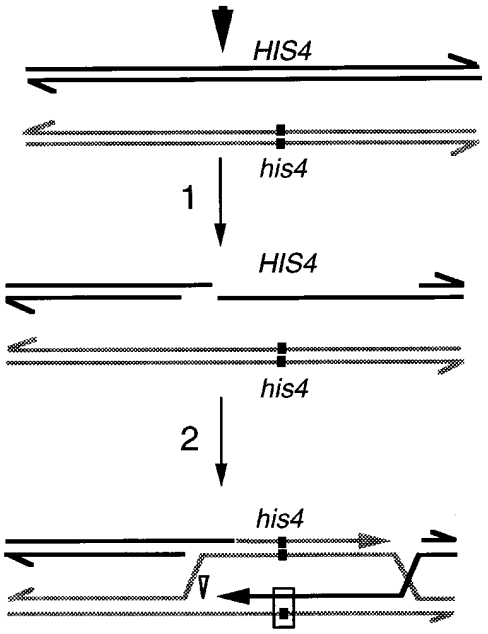

Figure 1.—Meiotic recombination at the *HIS4* locus. Recombination between one DNA molecule with a wild-type gene (dark lines) and one with a mutant gene (shaded line) is shown; the position of the mutant substitution is indicated by short vertical lines. As first shown at the *ARG4* locus (Sun *et al.* 1989, 1991), recombination at *HIS4* initiates with a double-strand DNA break upstream of the coding sequence (Fan *et al.* 1995); the break is shown by an arrow above the DNA molecule with the wild-type *HIS4* gene. At *HIS4*, this break appears to be processed asymmetrically (by 5′ to 3′ excision of one strand of the broken end) toward *HIS4* (as shown in Step 1 of this figure) or toward *BIK1*, a gene located on the opposite site of the break (Porter *et al.* 1993). Subsequently (Step 2), a heteroduplex is formed in which one DNA strand has wild-type and one has mutant information. The resulting mismatch is boxed. Arrows indicate gap-filling DNA synthesis. The inverted triangle shows the position of a DNA nick that could be used as a signal for conversion-type repair (Porter *et al.* 1993; Nicolas and Petes 1994).

In yeast and other fungi, the frequency of gene conversion declines from one end of the gene to the other (reviewed by Whitehouse 1984; Nicolas and Petes 1994). This pattern is called the polarity gradient. The high end of the polarity gradient is at the 5′ end of the *ARG4* and *HIS4* genes (Nicolas *et al.* 1989; Detloff and Petes 1992), but at the 3′ end of the *HIS2* gene (Malone *et al.* 1992, 1994). For all three genes, the high end of the gradient is adjacent to the site of the double-strand DNA break (DSB) that initiates recombination at the locus. In the context of the model shown in Figure 1, one explanation of the polarity gradient is that heteroduplexes are propagated from the DSB site to different extents as a consequence of different amounts of degradation of the broken DNA end. Thus, markers near the site of the initiating lesion will be included within the heteroduplex region more frequently than markers farther from the site. In support of this model, Sun *et al.* (1991) found that the DNA ends produced by the initiating DSB at the *ARG4* locus were degraded to variable extents in a pattern that mimicked the polarity gradient at *ARG4*.

There are two observations, however, that argue against the conclusion that the polarity gradient results solely from a gradient of heteroduplex formation. First, although the *HIS4* gene exhibits a polarity gradient for markers that lead to well-repaired mismatches (low-PMS alleles), this gradient is almost eliminated when markers that lead to poorly repaired mismatches (high-PMS alleles) are used (Detloff *et al.* 1992); in addition, the frequencies of aberrant segregation at the 5′ end of *HIS4* were approximately the same for high-PMS and low-PMS alleles, whereas, in the middle or at the end of the polarity gradient, the frequency of aberrant segregation was higher for the high-PMS alleles than for the low-PMS alleles (Detloff *et al.* 1992). Second, at both the *ARG4* and *HIS4* loci, the polarity gradient is substantially reduced by the *msh2* mutation, a mutation that eliminates DNA mismatch repair (Alani *et al.* 1994).

Two models were presented to explain these results. Detloff *et al.* (1992) suggested that the level of heteroduplexes was nearly constant from one end of *HIS4* to the other. They postulated that the polarity gradient observed for low-PMS markers reflected an alteration in the ratio of conversion-type to restoration-type repair as a function of the position of the mismatch relative to the initiating DSB. Mismatches near the initiating lesion were repaired exclusively by conversion-type repair, whereas mismatches located far away from the DSB site were repaired equally frequently by conversion-type and restoration-type repair, resulting in the twofold polarity gradient observed at the *HIS4* locus. Porter *et al.* (1993) suggested that the nick located near the 5′ end of *HIS4* (a consequence of the initiating DSB as shown in Figure 1) might direct the repair of nearby mismatches toward conversion-type repair. The ability of a nick to direct mismatch repair in a distance-dependent manner has been demonstrated *in vitro* (Modrich 1991), and nick- or gap-directed biases in mismatch repair have been observed for yeast mitotic recombination events initiated by a double-strand break (Haber *et al.* 1993; Sweetser *et al.* 1994). If the DNA mismatch is located far from the nick, the nick-directed bias would not operate, and conversion-type and restoration-type repair would be equally frequent. In subsequent discussions, we will call this model the R/C (restoration/conversion) model.

An alternative model—based partly on evidence that the DNA mismatch repair system in prokaryotes prevents recombination between diverged DNA sequences (Rayssiguier *et al.* 1989)—is that the polarity gradient reflects an interaction of DNA mismatches with the DNA mismatch repair enzymes during heteroduplex formation (Alani *et al.* 1994). Alani *et al.* (1994) showed that a mutation in the DNA mismatch repair gene *MSH2* greatly reduced the polarity gradient at the *HIS4* and

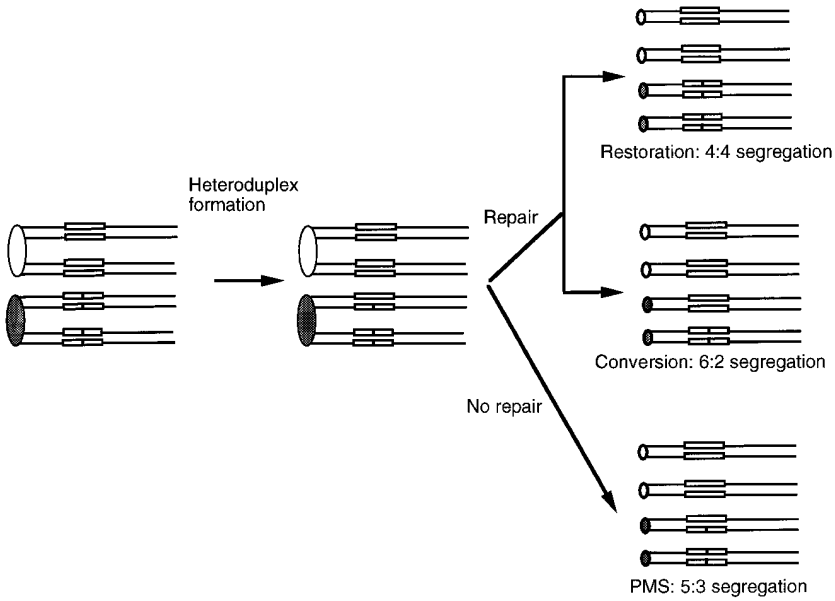

Figure 2.—Patterns of DNA mismatch repair. Heteroduplex formation between a wild-type gene (rectangle without vertical lines) and a mutant gene (rectangle with vertical lines) is shown. A DNA strand with wild-type information is nonreciprocally donated to a mutant gene. The resulting DNA mismatch can be repaired to restore the mutant gene (upper part of diagram) or to generate a wild-type gene (6:2 conversion event). Failure to repair the mismatch would result in a tetrad with a single PMS event (5:3 segregation). If heteroduplex formation involves nonreciprocal transfer of a mutant DNA strand to a wild-type gene, the comparable classes would be the following: 4:4 (restoration-type repair), 2:6 (conversion-type repair), and 3:5 (no repair).

ARG4 loci. They suggested that heteroduplex formation in yeast was initially asymmetric (nonreciprocal donation of a DNA strand from one chromosome to the other) but became symmetric (reciprocal exchange of strands between the two chromosomes) as a consequence of branch migration. Further, they argued that mismatches located in the region of symmetric heteroduplex resulted in reversal of the heteroduplex (removing the DNA mismatch) or termination of heteroduplex formation followed by symmetric repair of the mismatches. The net result of these processes would be a reduction in the level of aberrant segregation of markers displaced from the initiation site. Below, we will refer to this model as the H/A (heteroduplex/abortion) model.

In the experiments described below, we designed strains that allow us to estimate the frequency of restoration-type repair at two positions within the *HIS4* gene. We find that a mismatch located near the beginning of the gene undergoes less restoration-type repair than a mismatch located near the middle of the gene, consistent with the R/C model.

MATERIALS AND METHODS

Media: Standard media were used (Guthrie and Fink 1991). Sporulation plates contained 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 6 μg/ml adenine, and 2% agar.

Strains: Most strains were derived from the haploid strains AS13 (***a*** *leu2 ura3 ade6 rme1*) and AS4 (α *trp1 arg4 tyr7 ade6 ura3*) (Stapleton and Petes 1991) by two-step transplacement with plasmids containing various mutant *his4* alleles. The construction of most of the strains has been reported previously (Table 1). The haploid DTK287 was constructed by a two-step transplacement of AS4 with *Mfe*I-cut p65. This plasmid has a mutation in the initiation codon of *HIS4*, changing the ATG to ACG (Donahue and Cigan 1988). The crosses used to construct diploid strains and the *his4* alleles of these diploids are shown in Table 2.

The genotypes for allelism and complementation tester strains were the following: DTK172 (***a*** *his4-712 leu2 ade6 ura4*), DTK174 (α *his4-712 leu2 ura4*), DTK176 (α *his4-3133 leu2 ura4*), DTK178 (***a*** *his4-3133 leu2 ura4*), DTK282 (***a*** *his4-ACG leu2 ade6 ura4*), DTK280 (α his4-ACG leu2 ade6 ura4), PD21 (***a*** *his4-Δ29 leu2 ura3 ade6 rme1*), and PD68 (α *his4-Δ29 trp1 arg4 tyr7 ura3 ade6*).

Meiotic analysis: Diploids were sporulated on solid medium at 18° for 4 to 6 days, and tetrads were dissected onto plates containing the rich growth medium YPD by standard methods. After 3 days at 30°, the resulting spore colonies were replica-plated to diagnostic omission media. Because several of the *his4* alleles used in the study resulted in high levels of PMS, spore colonies replica-plated to medium lacking histidine were examined by light microscopy in order to detect small His^- sectors.

In diploid strains that were heterozygous for more than one *his4* mutant allele, we performed allelism or complementation tests using the tester strains described above. Details of these tests have been previously described (Detloff *et al.* 1992).

Statistical analysis: Instat 1.12 (GraphPad Software) for Macintosh was used for statistical tests. Fisher's exact variant of the chi-square test was used for most comparisons. The results were considered to be statistically different if $P < 0.05$.

RESULTS AND DISCUSSION

As described in the Introduction, if a heteroduplex formed during meiotic recombination includes strands derived from different alleles, the heteroduplex will contain mismatches. Although most base-base mismatches (with the exception of C/C) or mismatches with one or more displaced bases are efficiently repaired in wild-type yeast cells, mismatches that contain short palindromic loops are not readily repaired (Nag *et al.* 1989). Because lack of repair is associated with PMS events, we will refer to alleles that lead to poorly repaired mismatches as high-PMS alleles and those that result in

TABLE 1

Relevant genotypes of haploid strains

Strain	Reference	Relevant genotype[a]
AS13-derived		
AS13	Stapleton and Petes (1991)	*HIS4*
DNY47	Detloff and Petes (1992)	*his4-IR9*
PD6	White *et al.* (1992)	*his4-17*
PD21	Detloff and Petes (1992)	*his4-Δ29*
PD22	Detloff and Petes (1992)	*his4-712*
PD75	Detloff *et al.* (1992)	*his4-ACG*
PD98	Detloff *et al.* (1992)	*his4-3133*
PD100	Detloff and Petes (1992)	*his4-IR9 his4-3133*
AS4-derived		
AS4	Stapleton and Petes (1991)	*HIS4*
PD8	Detloff *et al.* 1991	*his4-17*
PD25	Detloff and Petes (1992)	*his4-712*
PD68	Detloff and Petes (1992)	*his4-Δ29*
DTK287	This study	*his4-ACG*

[a] All strains are isogenic with AS4 and AS13 except for changes introduced by transformation.

efficiently repaired mismatches as low-PMS alleles. Below, we describe the meiotic analysis of strains heterozygous for both high-PMS and low-PMS alleles within the *HIS4* gene. In the first study, a low-PMS allele is located between two high-PMS alleles. In the second study, we examine strains that have a low-PMS allele at the high end of the *HIS4* polarity gradient and a high-PMS allele at the low end of the gradient.

Demonstration of restoration-type repair: The strain DTK158 is heterozygous for three mutations within *HIS4* (Figure 3a). The locations of the mutations within the *HIS4* gene relative to the initiating codon are the following: +467 (*his4-IR9*), +1396 (*his4-712*), and +2327 (*his4-3133*). The *his4-712* allele is a frameshift mutation (insertion of G; Donahue *et al.* 1982) and is a low-PMS allele (Detloff *et al.* 1991), whereas the flanking alleles are 26-bp palindromic insertions behaving as high-PMS alleles (Detloff and Petes 1992). About two-thirds of the recombination events occurring within *HIS4* are initiated as a consequence of a double-strand DNA break in a region located about 100 bp upstream of *HIS4* (Detloff *et al.* 1992; Porter *et al.* 1993; Xu and Petes 1996). In addition, about two-thirds of the heteroduplexes initiated upstream of *HIS4* extend to the end of the *HIS4* gene (Detloff and Petes 1992; Porter *et al.* 1993).

From the considerations described above, we expect that about one-half ($2/3 \times 2/3$) of the recombination events initiated upstream of *HIS4* in DTK158 will result in heteroduplexes that contain all three heterozygous markers. Because the flanking markers are high-PMS alleles and the middle marker is a low-PMS allele, tetrads in which all three markers are included within a single heteroduplex would be expected to exhibit PMS segregation of the flanking markers (both 5:3 or both 3:5) and either gene conversion (as a consequence of conversion-type repair) or normal Mendelian segregation (as a consequence of restoration-type repair) of *his4-712*. One complication is that a tract of mismatch repair initiated at the low-PMS allele might include one of the flanking mismatches. Approximately one-third of excision tracts in yeast extend at least 900 bp from the mismatch (Detloff and Petes 1992). This effect is expected to reduce, but not eliminate, the number of diagnostic tetrads.

A summary of the frequencies and types of aberrant segregation for DTK158 is shown in Table 3. In addition, this table includes data from isogenic strains that were heterozygous for the individual mutant alleles. As expected, the *his4-IR9* and *his4-3133* alleles exhibited sub-

TABLE 2

Relevant genotypes of diploid strains

Strain[a]	Haploid parents[b]	Relevant genotype[c]
MD50	PD98 × PD8	*his4-3133/his4-17*
DTK158	PD100 × PD25	*his4-IR9 his4-3133/his4-712*
DTK289	PD98 × DTK287	*his4-3133/his4-ACG*
PD108	AS13 × PD25	*HIS4/his4-712*
PD99	PD98 × AS4	*his4-3133/HIS4*
PD85	PD75 × AS4	*his4-ACG/HIS4*
PD11	PD6 × AS4	*his4-17/HIS4*
DNY48	DNY47 × AS4	*his4-IR9/HIS4*

[a] References for diploid strains constructed in other studies are the following: PD108 (Detloff and Petes 1992), PD99 (Detloff *et al.* 1992), PD85 (Detloff *et al.* 1991), PD11 (White *et al.* 1992), and DNY48 (Nag and Petes 1991).

[b] All haploid strains are isogenic with AS4 and AS13, except for alterations introduced by tranformation. The first parents listed on each line are AS13 derivatives.

[c] The alleles listed above and below the lines are derived from AS13 and AS4 derivatives, respectively.

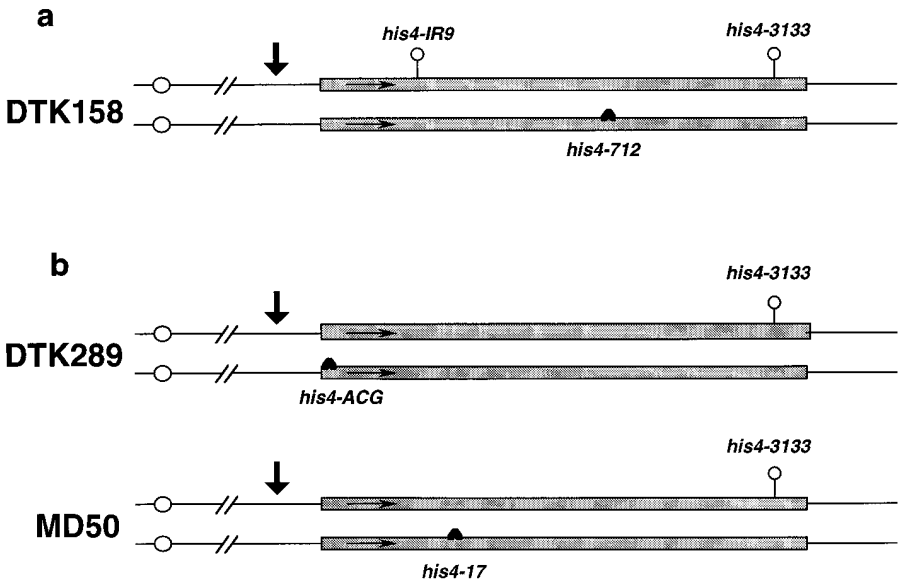

Figure 3.—Configuration of *his4* mutant alleles in strains DTK158, DTK289, and MD50. The *HIS4* coding sequences are shown by the rectangle, with the direction of transcription indicated by the horizontal arrows. The positions of the recombination-initiating double strand breaks are shown by the vertical arrows. (a) Arrangement of markers in DTK158. The *his4-IR9* and *his4-3133* alleles represent palindromic insertions (indicated by stem-loop structures) at positions +467 and +2327, respectively (+1 representing the first base in the initiating codon of *HIS4*). The *his4-712* allele (position +1396) is a frameshift mutation caused by insertion of a single G. (b) Arrangement of markers in strains DTK289 and MD50. The *his4-ACG* and *his4-17* mutations are T to C base changes located at positions +2 and +688, respectively.

stantially more PMS than *his4-712* in the triple mutant strain. The numbers of tetrads exhibiting PMS relative to conversion tetrads were reduced for *his4-IR9* and *his4-3133* in the triple mutant strains, compared to numbers in these classes in the single mutant strains DNY48 and PD99; the reduction was significant for *his4-IR9* (*P* value of 0.001 by Fisher exact test) but not for *his4-3133* (*P* value of 0.16). This reduction may reflect a fraction of tetrads in which the *his4-IR9* mismatch is corepaired with the *his4-712* mismatch. In addition, the levels of aberrant segregation for the *his4-IR9* and *his4-3133* alleles were not significantly altered in the triple mutants relative to the single mutants (*P* values >0.05). Although the *his4-712* marker is a low-PMS allele in DTK158, it shows significantly more PMS in DTK158 than it does in the single mutant strain PD108. Although the interpretation of this effect is not clear, it has been previously noted that high-PMS alleles can increase the PMS frequencies of nearby low-PMS alleles in yeast (Detloff and Petes 1992; Manivasakam *et al.* 1996).

We analyzed 321 tetrads from DTK158. The patterns of segregation at the three heterozygous *his4* markers are shown in Figure 4. We group the data into tetrads in which the aberrant segregation patterns are explicable by a single event (Figure 4a) and those requiring multiple independent events (Figure 4b). For single-event tetrads, we assume the following: (1) heteroduplex formation is asymmetric, (2) conversion tracts are continuous, and (3) crossovers occur at the end of the heteroduplex. The validity of these assumptions will be discussed further below.

The classes of tetrads that allow an estimation of the relative frequencies of conversion-type and restoration-type repair for the *his4-712* allele are those in which a single spore exhibits PMS for the flanking palindromic markers, but either normal Mendelian segregation or

TABLE 3

Number of tetrads with various segregation patterns for *his4* mutant alleles in strains DNY48, PD108, PD99, and DTK158[a]

Strain	Allele	4:4	6:2	2:6	5:3	3:5	Ab 4:4	Other PMS	Other non-PMS	Total	Ab Seg (%)	PMS/Ab Seg. (%)
DNY48[b]	*his4-IR9*	240	15	15	50	44	8	6	1	379	37	78
PD108[b]	*his4-712*	157	13	27	0	1	0	0	2	200	22	2
PD99[b]	*his4-3133*	218	22	19	33	39	6	6	1	344	37	67
DTK158	*his4-IR9*	184	20	32	28	43	3	7	4	321	43	59
	his4-712	224	23	49	10	12	0	1	2	321	30	24
	his4-3133	226	23	16	16	31	4	3	2	321	30	57

[a] For all segregation patterns, the first and second numbers represent the wild-type and mutant alleles, respectively. The classes include the following: 4:4 (normal Mendelian segregation), 6:2 and 2:6 (gene conversion), 5:3 and 3:5 (tetrads with a single PMS event), and Ab 4:4 (aberrant 4:4; one wild-type, one mutant, and two sectored colonies). The "Other PMS" class includes aberrant 6:2, 2:6, 7:1, 1:7 tetrads and tetrads with three or four PMS events; the "Other non-PMS" class consists of 8:0 and 0:8 tetrads.

[b] Detloff and Petes (1992).

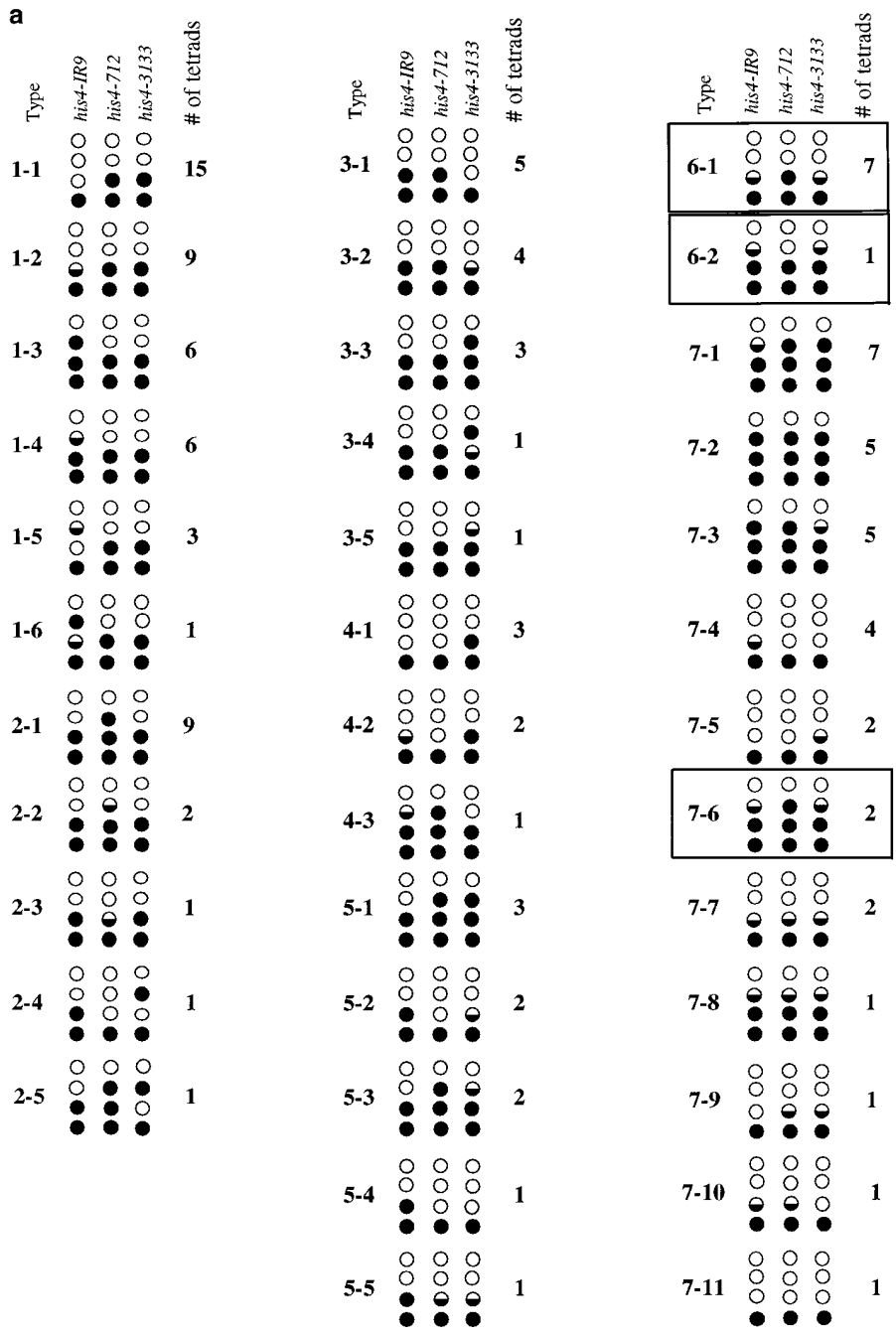

Figure 4.—Patterns of gene conversion and postmeiotic segregation in tetrads derived from DTK158. For each type of tetrad, we show three columns (each column representing the segregation pattern of one mutant allele) with four circles (each representing a spore colony) in each column. Each row represents the genotype of a single spore of the tetrad. For the *his4-IR9* and *his4-3133* columns, an open circle indicates a spore colony with the mutant allele and a solid circle indicates a spore colony with the wild-type allele. For the *his4-712* column, an open circle indicates a spore colony with a wild-type allele and a solid circle indicates a spore colony with a mutant allele. Thus, a tetrad in which no recombination events at the *his4* locus occurred would be depicted by two rows of open circles (parental configuration of the "top" chromosome in Figure 3a) and two rows of solid circles (parental configuration of the "bottom" chromosome in Figure 3a). Circles that are half open and half closed indicate a postmeiotic segregation event for the *his4* allele. (a) Single-event tetrads. We depict those patterns of meiotic segregation that can be explained by single events by the following assumptions: (1) heteroduplex formation is asymmetric, (2) gene conversion tracts are continuous, and (3) crossovers occur at the ends of heteroduplexes. For example, the Class 1-5 tetrad can be explained by a single heteroduplex that included only the *his4-IR9* allele with a crossover between *his4-IR9* and *his4-712*. The classes of tetrads that are most relevant to our analysis of restoration-type and conversion-type repair are shown in boxes. (b) Multiple-event tetrads. These classes represent tetrads that require more than one asymmetric heteroduplex, discontinuous mismatch repair, and/or a crossover that is not associated with the end of a heteroduplex. As discussed in the text, a small fraction of these tetrads, *e.g.*, Class 8-7 could be explained by formation of single symmetric heteroduplexes, rather than by two independent events.

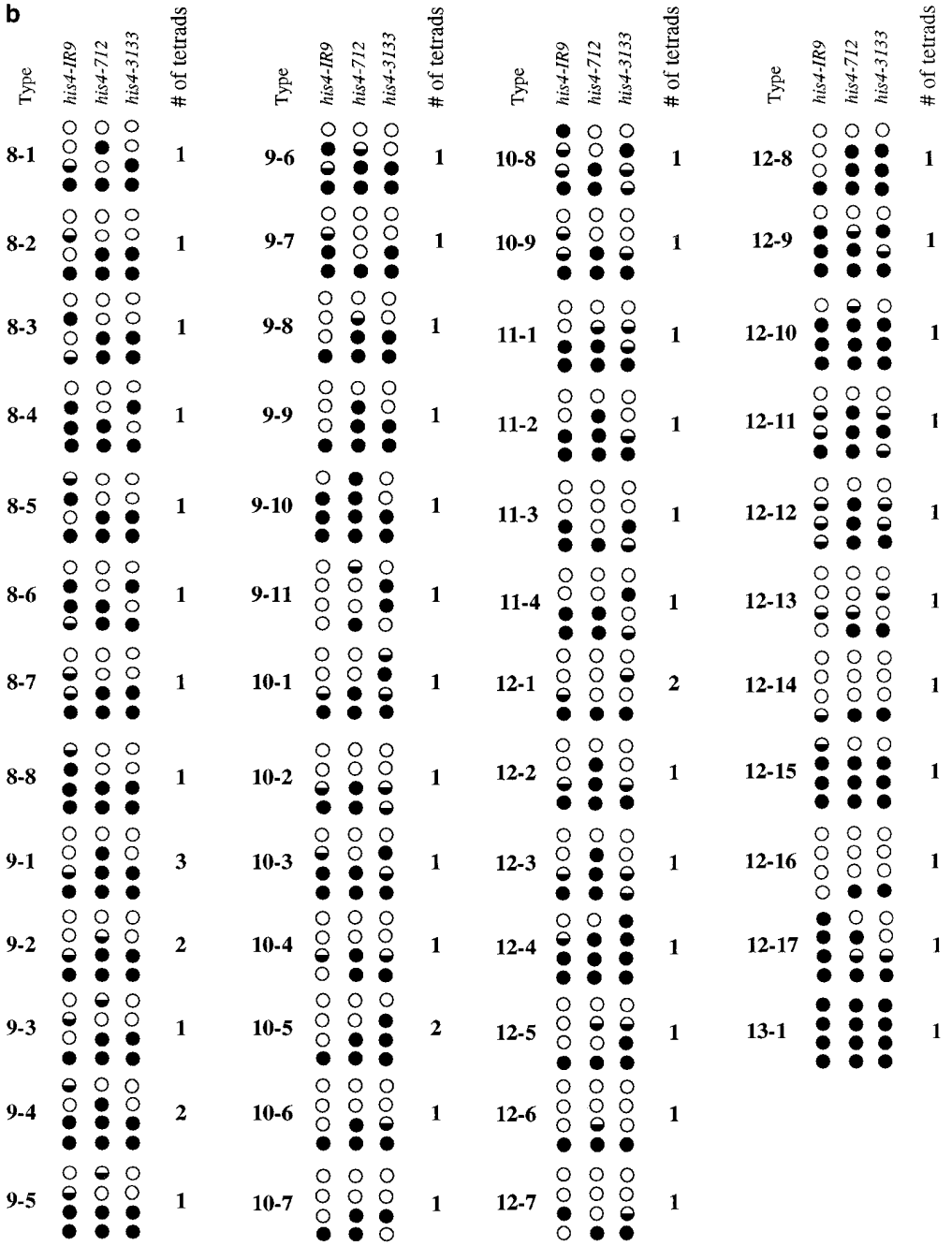

Figure 4.—*Continued.*

gene conversion for the middle marker. In addition, the gene conversion event must have a pattern consistent with a single heteroduplex involving all three alleles. Thus, if the flanking markers segregate 5:3, the gene conversion event must be 6:2 rather than 2:6. There are 10 tetrads with these patterns (Classes 6-1, 6-2, and Class 7-6). In 8 of those 10 tetrads, the presumptive mismatch with the *his4-712* allele underwent restoration-type repair and, in two tetrads, conversion-type repair was observed. Although the number of tetrads in these classes is too small to obtain an accurate estimate of the ratio of the two types of repair at this position in *HIS4*, the results clearly indicate the existence of restoration-type repair in *S. cerevisiae*.

An alternative explanation of the classes of tetrads that appear to exhibit restoration-type repair is that they represent two independent events, one involving only the 5′ high-PMS marker and one involving only the 3′ high-PMS marker. Although tetrads in which the 5′ and 3′ markers underwent independent aberrant segregation events and the middle marker segregated 4:4 were observed (Class 10 in Figure 4b, representing 10 of 321 tetrads), the likelihood of independent events giving rise to seven Class 6-1 tetrads can be calculated to be very low. There were nine tetrads (Class 1-2) that had 3:5 segregation for *his4-IR9* and normal Mendelian segregation for the other markers and no associated crossover. There were four tetrads (Class 3-2) with 3:5 segre-

gation for *his4-3133* and normal Mendelian segregation for the other markers and no associated crossover. The expected frequency of Class 6-1 tetrads formed as a consequence of two events is about: (%Class 1-2 + %Class 6-1) × (%Class 3-2 + %Class 6-1), or 0.0016. If the events involving the two flanking markers were independent, crossovers associated with heteroduplex formation (which would be expected to occur between the flanking markers and the middle marker) would further reduce the number of Class 6-1 tetrads. Consequently, we conclude that it is very unlikely that Class 6-1 tetrads reflect formation of two independent heteroduplexes.

Two other arguments in favor of our interpretation can be made. First, tetrads with independent events involving the two palindromic insertions should sometimes result in 5:3 segregation for *his4-IR9* and 3:5 segregation for *his4-3133*. No tetrads with this pattern were observed. Second, if tetrads in which both palindromic markers segregated 5:3 or 3:5 represent independent events, then half of those tetrads should involve two different spores rather than the same spore. In all eight tetrads in which the flanking markers segregated 5:3 or 3:5 and *his4-712* exhibited normal Mendelian segregation, the same spore underwent PMS.

A second possible artifact that could account for the Class 6 restoration-type tetrads is that the sector resulting from unrepaired mismatch involving *his4-712* was not detected by the allelism test (described in materials and methods), although allelism tests detected sectors for the flanking markers. To eliminate this possibility, we examined in more detail four spore colonies derived from four Class 6-1 tetrads. In Class 6-1 tetrads, one spore colony is sectored for the flanking markers but is not sectored for the *his4-712* marker. Such colonies derived from the original dissection plate were streaked on rich growth medium to generate multiple single colonies derived from the sectored colony. The allelism test was repeated on at least 25 colonies derived from each sectored spore colony. For all 4 spore colonies examined, this test confirmed the presence of two genotypes for the flanking markers and the presence of a single genotype for the middle marker. This result suggests that the lack of observed sectoring for *his4-712* is unlikely to represent a failure of the allelism test to detect a small sector.

In summary, the results obtained with DTK158 provide direct evidence for restoration-type repair in *S. cerevisiae* and are consistent with previous indirect evidence for this mode of repair in yeast (Hastings 1984; Detloff *et al.* 1992). Using the same method employed in our study, Hastings *et al.* (1980) previously showed the existence of restoration-type repair in *A. immersus*.

As in previous studies of aberrant segregation at the *HIS4* recombination hotspot, we found some tetrads (21/321, 7%) in which one or more of the markers had an aberrant segregation pattern different from 6:2, 2:6, 5:3, or 3:5. Such tetrads could represent meioses in which more than one initiating event occurred or single events in which one or more of the assumptions about the nature of single events described above was violated. For example, a tetrad with an aberrant 4:4 segregation (one wild-type, one mutant, and two sectored spore colonies) could represent independent formation of two asymmetric heteroduplexes or a single event generating a symmetric heteroduplex. In some models of recombination (Meselson and Radding 1975; Szostak *et al.* 1983), symmetric heteroduplexes result from branch migration of a structure that initially has only a single region of heteroduplex, whereas in other models (Holliday 1964) the recombination-initiating event results in symmetric heteroduplexes. In *S. cerevisiae*, aberrant 4:4 tetrads are generally observed at a frequency expected for two asymmetric heteroduplexes (Fogel *et al.* 1981; Nag *et al.* 1989; Nag and Petes 1990). The simplest interpretation of this result is that heteroduplex formation in yeast is primarily asymmetric, although it has been suggested that a fraction of the recombination events observed at the 3′ end of *HIS4* reflect symmetric heteroduplex formation (Alani *et al.* 1994).

In DTK158, we found seven tetrads with aberrant 4:4 segregation for either *his4-IR9* or *his4-3133* (Figure 4b). It is likely that these events represent two independent initiation events for two reasons. First, the frequency of these events is low enough to represent two independent events. For example, the frequencies of 5:3, 3:5, aberrant 6:2, aberrant 2:6, and aberrant 4:4 segregation for *his4-IR9* were 9%, 13%, 0.3%, 0%, and 1%, respectively. The predicted frequency of aberrant 4:4 tetrads can be calculated to be the following: 2 [%5:3 + %Ab. 6:2 + (1/2)%Ab. 4:4] × [%3:5 + %Ab. 2:6 + (1/2) %Ab. 4:4], or 2.7%. This calculation is based on the assumption that each chromatid can receive and donate information only once at the *his4-IR9* site and that the same chromatid cannot donate and receive information in consecutive events. If these restrictions are relaxed, the expected frequency of aberrant 4:4 tetrads is halved to 1.4%. The observed frequency of aberrant tetrads is 1%, close to the values expected as a consequence of two independent asymmetric heteroduplexes.

A second argument that the aberrant 4:4 tetrads are unlikely to reflect a single symmetric heteroduplex is that we found aberrant segregation patterns (aberrant 6:2, aberrant 2:6, 7:1, 1:7) explicable by two asymmetric heteroduplexes, but not by a single symmetric heteroduplex, at frequencies similar to the frequency of aberrant 4:4 tetrads (Table 3 and Figure 4b). Although the 8:0 and 0:8 classes of tetrads may reflect two independent meiotic gene conversion events or a mitotic conversion event, the frequencies of these classes suggest that they probably reflect double meiotic events. In summary, most of the aberrant segregation events observed

in DTK158 are most simply explained as reflecting processing of one or more asymmetric heteroduplexes.

Meiotic segregation patterns in strains that have a low-PMS allele at the high end of the *HIS4* polarity gradient and a high-PMS allele at the low end of the gradient: As discussed in the Introduction, the polarity gradients observed for low-PMS alleles at the *HIS4* and *ARG4* loci in *S. cerevisiae* involve the DNA mismatch repair system. Two different possible roles of this system have been suggested: (1) the ratio of conversion-type to restoration-type repair is higher for DNA mismatches located near the initiation site of recombination (R/C model of Detloff *et al.* 1992) and (2) heteroduplex propagation is reversed or aborted by DNA mismatch-repair enzymes acting on DNA mismatches; this effect is dependent on the location of the mismatch relative to the initiation site for heteroduplex formation (H/A model of Alani *et al.* 1994). One distinction between the two models is that the R/C model predicts a difference in the level of gene conversion for low-PMS alleles, even in tetrads in which the heteroduplex extends to the end of the *HIS4* gene. The strains DTK289 and MD50 were constructed to test this prediction.

Strains DTK289 and MD50 are heterozygous for the palindromic *his4-3133* marker located near the 3′ end of *HIS4* and heterozygous for low-PMS alleles located near the 5′ end of *HIS4* (Figure 3b). Both low-PMS alleles represent the same single base-pair alteration. DTK289 is heterozygous for *his4-ACG* located at position +2, and MD50 is heterozygous for *his4-17* located at position +688. In previous studies, the patterns of aberrant segregation for isogenic strains singly heterozygous for each of these mutations have been examined (Detloff *et al.* 1991; Detloff and Petes 1992; White *et al.* 1992), and these data are summarized in Table 4. The segregation patterns are also depicted in Figure 5. As expected from analysis of the single mutant strains, the frequency of aberrant segregation for *his4-ACG* in DTK289 was significantly ($P < 0.0001$) higher than that seen for *his4-17* in MD50, consistent with the polarity gradient detected previously at *HIS4* (Detloff *et al.* 1992).

We first examined the frequencies of gene conversion and normal Mendelian segregation of the upstream marker (*his4-ACG* or *his4-17*) in tetrads with a single PMS event or conversion for *his4-3133*. Because most (about two-thirds) heteroduplexes that involve markers near the 3′ end of *HIS4* are initiated at the hotspot located near the 5′ end of the gene (Detloff and Petes 1992), such tetrads are likely to reflect formation of a single heteroduplex that includes both markers with conversion-type or restoration-type repair of mismatches involving the upstream marker. As shown in Figure 5, in DTK289, 14 tetrads (Classes 2-1 to 2-4) had a single conversion or PMS event at *his4-3133* associated with normal Mendelian segregation at *his4-ACG*. Twenty tetrads (Classes 3-1 to 3-4) had a single conversion event

TABLE 4

Number of tetrads with various segregation patterns for *his4* mutant alleles in strains PD85, PD11, PD99, DTK289, and MD50

Strain	Allele	4:4	6:2	2:6	Other non-PMS	5:3	3:5	Ab 4:4	Other PMS	Total	Ab Seg (%)	GC (%)	PMS (%)	PMS/AbSeg (%)
PD85[a]	*his4-ACG*	413	132	156	37	12	25	2	7	784	47	42	6	12
PD11[b]	*his4-17*	184	22	40	4	6	12	1	0	269	32	25	7	22
PD99[c]	*his4-3133*	218	22	19	1	33	39	6	6	344	37	12	24	67
DTK289	*his4-ACG*	124	41	47	13	10	12	0	3	250	50	40	10	20
	his4-3133	165	7	7	0	24	27	12	8	250	34	6	28	84
MD50	*his4-17*	284	35	36	0	14	16	5	2	392	28	18	9	34
	his4-3133	262	26	25	0	29	43	4	3	392	33	13	20	61

The segregation patterns are depicted in the manner explained in Table 3. "GC" indicates gene conversion (6:2 or 2:6).

[a] Detloff *et al.* (1991).

[b] White *et al.* (1992) (rescored from original data).

[c] Detloff and Petes (1992).

or PMS event at *his4-3133* associated with gene conversion at *his4-ACG*. Classes of tetrads with a crossover between the two markers, *e.g.*, Class 2-5, are not likely to represent a single heteroduplex involving both markers and were not included in our calculations. In the strain MD50 (Figure 5), we found 62 tetrads with normal Mendelian segregation for *his4-17* and associated PMS or conversion at *his4-3133* (Classes 2-1 to 2-4) and 27 tetrads with gene conversion of *his4-17* and associated PMS or conversion at *his4-3133* (Classes 3-1 to 3-4).

In those tetrads with a single aberrant segregation event at *his4-3133*, a significantly ($P < 0.01$) higher fraction of tetrads of Classes 2-1 to 2-4 (pattern expected for restoration-type repair of the upstream mismatch) were found for MD50 compared to DTK289. The simplest interpretation of this result is that mismatches involving the marker located furthest from the site of DSB formation (*his4-17*) undergo more restoration-type repair than mismatches involving the marker (*his4-ACG*) located closer to the DSB site, consistent with the R/C model.

It should be pointed out that some of the tetrads in Classes 2-1 to 2-4 may involve heteroduplex formation that includes only *his4-3133*. Because about one-half of such tetrads would be expected to have a crossover between *his4-3133* and the upstream marker (such as observed for Class 2-5 and 2-6 tetrads), we conclude that these types of events are relatively rare. In addition, one would expect that the number of such tetrads would be the same in DTK289 and MD50 and therefore would not result in a significant difference in the numbers of diagnostic tetrads in the two strains.

A second test of the models involves a comparison of the levels of aberrant segregation of *his4-3133* in a strain with no other *his4* mutations (PD99) and in strains with an upstream low-PMS marker (DTK289 and MD50). If the R/C model is correct, the low-PMS alleles in strains DTK289 and MD50 will have little effect on the aberrant segregation frequency of the high-PMS allele at the low end of the conversion gradient. Thus, this model predicts that the level of aberrant segregation of *his4-3133* will be about the same in strains PD99, DTK289, and MD50.

In the H/A model, well-repaired mismatches (a consequence of heteroduplex formation involving low-PMS alleles) will cause reversal of heteroduplex formation or termination of heteroduplex formation; this effect occurs with low efficiency at the high-end of the conversion gradient but high efficiency near the low-end of the gradient (Alani *et al.* 1994). Thus, a prediction of this model is that a low-PMS allele near the middle of the conversion gradient will reduce the level of aberrant segregation frequency of a marker at the low end of the gradient, whereas a low-PMS allele at the high-end of the gradient will have little effect. Consequently, if the H/A model is correct, we expect a lower aberrant segregation frequency for *his4-3133* in strain MD50 than in

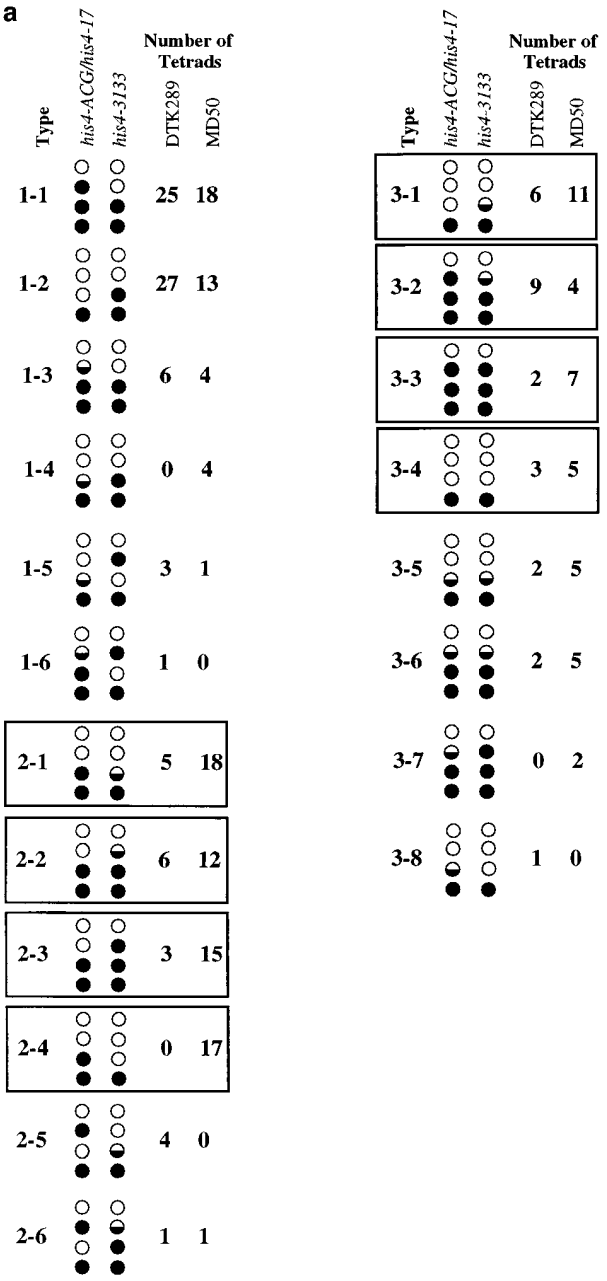

Figure 5.—Patterns of gene conversion and postmeiotic segregation in tetrads derived from DTK289 and MD50. The depiction of aberrant segregation patterns is similar to that shown in Figure 4. Open and solid circles represent mutant and wild-type colonies, respectively, for *his4-3133*, and, for the upstream markers, open and solid circles represent wild-type and mutant colonies, respectively. As in Figure 4, we assume that heteroduplex formation is asymmetric and continuous and that the crossover event occurs at the end of the heteroduplex. Boxed patterns indicate tetrads used to calculate the relative frequencies of restoration-type and conversion-type repair for the upstream *HIS4* marker in the two strains, as discussed in the text. (a) Single-event and (b) multiple-event tetrads.

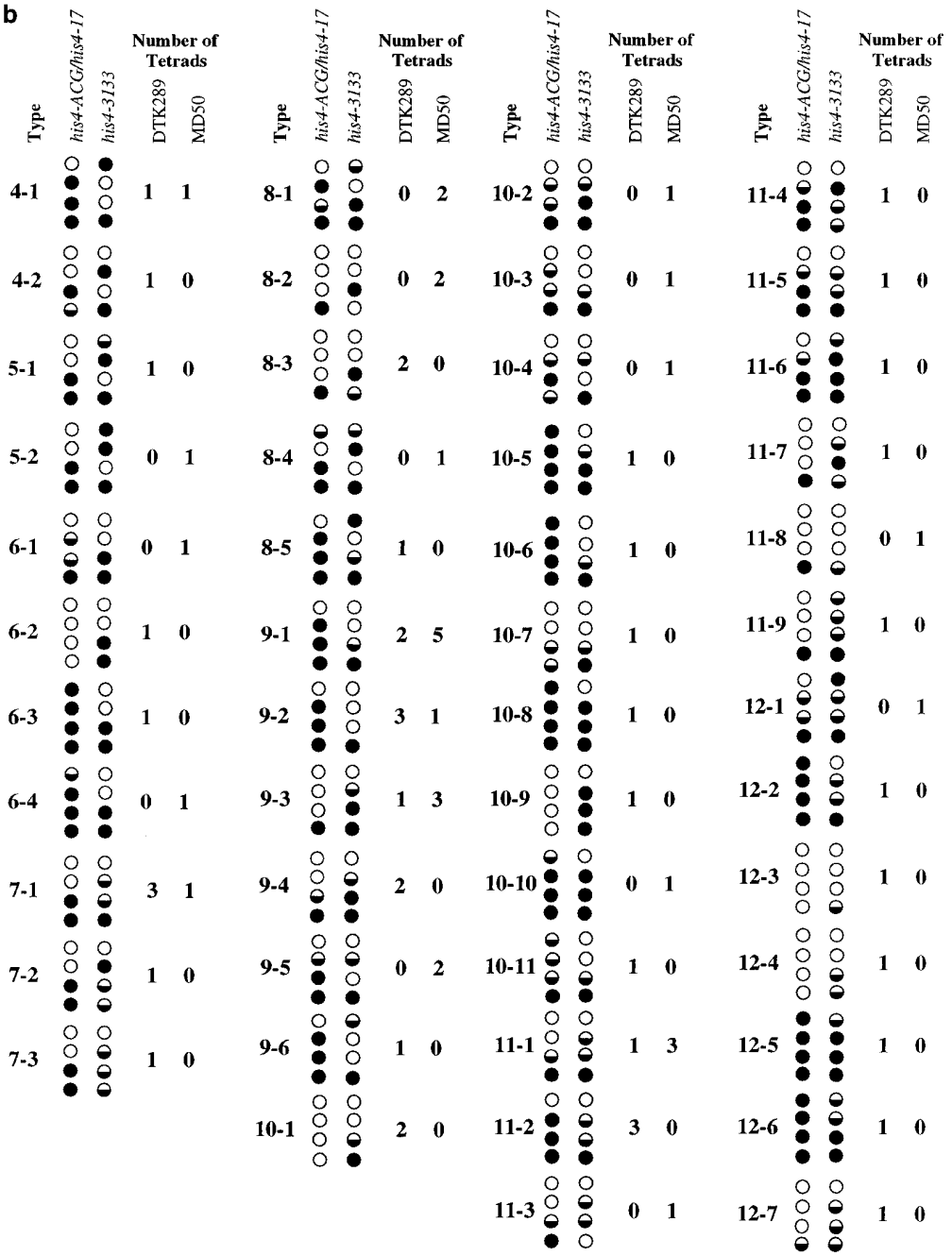

Figure 5.—*Continued.*

strains PD99 or DTK289. In a previous test of this expectation using strains isogenic with PD99 and MD50, Alani *et al.* (1994) found that heterozygosity for the *his4-17* marker reduced the level of aberrant segregation of *his4-3133* from 28 to 21%, an effect that was statistically significant.

In the studies of Alani *et al.* (1994), strains were sporulated at 30°. Our previous studies (Nag *et al.* 1989; Detloff *et al.* 1992) involved sporulation at 18°, a condition that results in high levels of recombination at the *HIS4* locus. Under these conditions, we find that the levels of aberrant segregation of *his4-3133* in the double mutant strains were not significantly different from those observed in the single mutant strains (all comparisons by Fisher exact test, $P > 0.1$). In addition, although the aberrant segregation frequency of *his4-3133* was slightly greater in PD99 (37%) compared to those observed in strains DTK289 (34%) and MD50 (33%), there was no significant difference in the segregation frequency between DTK289 and MD50, in contrast to the expectation of the H/A model. A slight reduction in the aberrant segregation frequency of *his4-3133* in strains DTK289 and MD50 relative to PD99 may reflect a failure to detect a small fraction of sectored colonies in these strains, because the detection requires allelism tests. In summary, a well-repaired mismatch located at the 5′

end (*his4-ACG*) or toward the middle (*his4-17*) of the polarity gradient does not impede heteroduplex formation at the 3′ end of the gradient. It is unclear whether the difference between our results and those of Alani *et al.* (1994) represents an effect of sporulation temperature or some other differences in the procedures of sporulation and the analysis of the genotypes of spore colonies. It is also possible that some of the basic properties of meiotic recombination, *e.g.*, the length of heteroduplexes or direction of mismatch repair, are affected by the temperature of sporulation; in the genetic background used in our studies, meiotic recombination events at the *HIS4* locus are initiated more frequently when the cells are sporulated at low temperatures (Fan *et al.* 1995).

As described previously, in current models of recombination, gene conversion events result from repair of mismatches in heteroduplexes, and PMS events reflect unrepaired mismatches. In DTK289, 20% of the aberrant segregation events involving *his4-ACG* were PMS, whereas 34% of the aberrant segregation events involving *his4-17* were PMS; these differences are statistically significant ($P = 0.02$). Because the mutant substitution is the same for these two alleles, one would expect the same type of DNA mismatch to be generated. There are two possible explanations for the difference in the ratio of conversion to PMS tetrads for these two alleles. First, the efficiency of repair of the mismatch could vary according to its position in the polarity gradient and/or its sequence context. An alternative explanation is that most of the mismatches involving *his4-ACG* are corrected by conversion-type repair, whereas those involving *his4-17* are corrected by both conversion-type and restoration-type repair. By this pattern of correction, one would find a higher fraction of PMS/aberrant segregants for *his4-17* without an effect on the efficiency of DNA mismatch repair. It should be noted that the percentage of tetrads exhibiting PMS at *his4-ACG* (10%) in DTK289 is about the same as that observed for *his4-17* (9%) in MD50, consistent with the second explanation.

Conclusions: In summary, we find evidence for restoration-type repair in *S. cerevisiae* and results indicating that the ratio of conversion-type to restoration-type repair contributes to the *HIS4* polarity gradient. In their analysis of *HIS4* recombination in various mismatch repair-deficient strains, Hunter and Borts (1997), reached a different conclusion. They found that *mlh1* strains, unlike those with an *msh2* mutation (Alani *et al.* 1994), had elevated frequencies of aberrant segregation, even for markers located near the high end of the *HIS4* polarity gradient (Hunter and Borts 1997); in addition, the polarity gradient was not eliminated in *mlh1* strains. These results indicate that, at least in *mlh1* strains, there is a gradient of heteroduplex formation, and Hunter and Borts (1997) suggest that the Mlh1p may be involved in limiting the length of the heteroduplex. One interpretation consistent with these results is that Mlh1p may be involved in reducing heteroduplex formation immediately adjacent to the recombination-initiation site and that the aberrant heteroduplexes that occur in the *mlh1* background are limited by an Msh2p-dependent mechanism (possibly, mismatch-triggered abortion of heteroduplex extension). An alternative possiblity is that Mlh1p (but not Msh2p) may be involved in a type of restoration repair that is independent of the location of the DNA mismatch in the polarity gradient. A final possibility is that the mechanism by which polarity gradients are formed can be different in different genetic backgrounds and sporulation conditions.

Our conclusion needs to be qualified in several other ways. First, our explanations of aberrant segregation patterns involve certain simplifying assumptions. For example, if a single heteroduplex involves the flanking markers, we assume that it also includes the middle marker. If recombination occurs by a mechanism that allows coordinated but discontinuous strand transfer, our conclusions would not be valid. Second, a polarity gradient in which mismatches at the high end are repaired to generate gene conversion events exclusively, and at the low end are repaired with equal frequency to conversion and restoration, will produce only a twofold gradient (such as observed at *HIS4*). Steeper gradients, such as observed at the *ARG4* locus in yeast (Fogel *et al.* 1981), must result either from preferential repair to restoration at the low end of the gradient or from another mechanism. Third, physical evidence for a gradient of heteroduplex formation has been reported at the *ARG4* locus (Sun *et al.* 1991). Fourth, the frequency of heteroduplex formation in some recombination systems is significantly affected by DNA mismatches (reviewed by Alani *et al.* 1994). In addition, single DNA mismatches can affect the frequency of mitotic (Datta *et al.* 1997) and meiotic (J. Lacombe, B. deMassy, A. Nicolas and E. Alani, personal communication) recombination in yeast. Fifth, evidence consistent with the formation of polarity gradients by a switch from asymmetric to symmetric heteroduplexes has been obtained in yeast (Alani *et al.* 1994; J. Lacombe, B. deMassy, A. Nicolas and E. Alani, personal communication).

Given these considerations, it is possible (and perhaps even likely) that polarity gradients can be formed by multiple mechanisms and that the relative importance of these mechanisms may show organism-to-organism and locus-to-locus variation. As suggested previously (Nicolas and Petes 1994), a steep gradient, such as that observed at *ARG4*, may represent the superimposition of two or more mechanisms that, by themselves, would result in only shallow gradients.

We thank D. Nag and T. Donahue for providing plasmids used in the study and F. Stahl and E. Alani for comments on the manuscript. The research was supported by National Institutes of Health grant GM-24110.

LITERATURE CITED

Alani, E., R. A. G. Reenan and R. D. Kolodner, 1994 Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. Genetics **137:** 19–39.

Datta, A., M. Hendrix, M. Lipsitch and S. Jinks-Robertson, 1997 Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc. Natl. Acad. Sci. USA **94:** 9757–9762.

Detloff, P., and T. D. Petes, 1992 Measurements of excision repair tracts formed during meiotic recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1805–1814.

Detloff, P., 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.

Detloff, P., M. A. White and T. D. Petes, 1992 Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. Genetics **132:** 113–123.

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.

Donahue, T. F., P. J. Farabaugh and G. R. Fink, 1982 The nucleotide sequence of the *HIS4* region of yeast. Gene **18:** 47–59.

Fan, Q.-Q., F. Xu and T. D. Petes, 1995 Meiosis-specific double-strand DNA breaks at the *HIS4* recombination hot spot in the yeast *Saccharomyces cerevisiae*: control in *cis* and *trans*. Mol. Cell. Biol. **15:** 1679–1688.

Fogel, S., R. K. Mortimer and K. Lusnak, 1981 Mechanisms of meiotic gene conversion, or "Wandering on a foreign strand," pp. 289–393 in *The Molecular Biology of the Yeast Saccharomyces*, Vol. 1, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Guthrie, C., and G. R. Fink (Editors), 1991 *Guide to Yeast Genetics and Molecular Biology*. Academic Press, San Diego.

Haber, J. E., B. L. Ray, J. M. Kolb and C. I. White, 1993 Rapid kinetics of mismatch repair of heteroduplex DNA that is formed during recombination in yeast. Proc. Natl. Acad. Sci. USA **90:** 3363–3367.

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.

Hastings, P. J., A. Kalogeropoulos and J.-L. Rossignol, 1980 Restoration to the parental genotype of mismatches formed in recombinant DNA heteroduplex. Curr. Genet. **2:** 169–174.

Holliday, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **5:** 282–304.

Hunter, N., and R. H. Borts, 1997 Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. Genes Dev. **11:** 1573–1582.

Malone, R. E., S. Bullard, S. Lundquist, S. Kim and T. Tarkowski, 1992 A meiotic gene conversion gradient opposite to the direction of transcription. Nature **359:** 154–155.

Malone, R. E., S. Kim, S. A. Bullard, S. Lundquist, L. Hutchings-Crow *et al.*, 1994 Analysis of a recombination hotspot for gene conversion occurring at the *HIS2* gene of *Saccharomyces cerevisiae*. Genetics **137:** 5–18.

Manivasakam, P., S. M. Rosenberg and P. J. Hastings, 1996 Poorly repaired mismatches in heteroduplex DNA are hyper-recombinagenic in *Saccharomyces cerevisiae*. Genetics **142:** 407–416.

Meselson, M. S., and C. M. Radding, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

Modrich, P., 1991 Mechanisms and biological effects of mismatch repair. Annu. Rev. Genet. **25:** 229–253.

Nag, D. K., and T. D. Petes, 1990 Genetic evidence for preferential strand transfer during meiotic recombination in yeast. Genetics **125:** 753–761.

Nag, D. K., and T. D. Petes, 1991 Seven-base-pair inverted repeats in DNA form stable hairpins *in vivo* in *Saccharomyces cerevisiae*. Genetics **129:** 669–673.

Nag, D. K., and T. D. Petes, 1993 Physical detection of heteroduplexes during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 2324–2331.

Nag, D. K., M. A. White and T. D. Petes, 1989 Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature **340:** 318–320.

Nicolas, A., and T. D. Petes, 1994 Polarity of meiotic gene conversion in fungi: contrasting views. Experientia **50:** 242–252.

Nicolas, A., D. Treco, N. P. Schultes and J. W. Szostak, 1989 An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–39.

Petes, T. D., R. E. Malone and L. S. Symington, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis, and Energetics*, Vol. 1, edited by J. R. Broach, J. R. Pringle and E. W. Jones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Porter, S. E., M. A. White and T. D. Petes, 1993 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. Genetics **134:** 5–19.

Rayssiguier, C., D. S. Thaler and M. Radman, 1989 The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature **342:** 396–400.

Stahl, F., 1996 Meiotic recombination in yeast: coronation of the double-strand-break repair model. Cell **87:** 965–968.

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.

Sun, H., D. Treco, N. P. Schultes and J. W. Szostak, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

Sun, H., D. Treco and J. W. Szostak, 1991 Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **64:** 1155–1161.

Sweetser, D. B., H. Hough, J. F. Whelden, M. Arbuckle and J. A. Nickoloff, 1994 Fine-resolution mapping of spontaneous and double-strand break-induced gene conversion tracts in *Saccharomyces cerevisiae* reveals reversible mitotic conversion polarity. Mol. Cell. Biol. **14:** 3863–3875.

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.

White, M. A., P. Detloff, M. Strand and T. D. Petes, 1992 A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the *HIS4* locus in yeast. Curr. Genet. **21:** 109–116.

Whitehouse, H. L. K., 1984 *Genetic Recombination*, John Wiley & Sons, New York.

Xu, F., and T. D. Petes, 1996 Fine-structure mapping of meiosis-specific double-strand DNA breaks at a recombination hotspot associated with an insertion of telomeric sequences upstream of the *HIS4* locus in yeast. Genetics **143:** 1115–1125.

Communicating editor: M. Lichten